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THE ABSOLUTE CONFIGURATION OF STREPTIDINE IN STREPTOMYCIN

A THESIS

Presented to

The Faculty of the Graduate Division

by

Aaron William Todd

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

in the School of Chemistry

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THE ABSOLUTE CONFIGURATION OF STREPTIDINE

IN STREPTOMYCIN

Approved:

[redacted]

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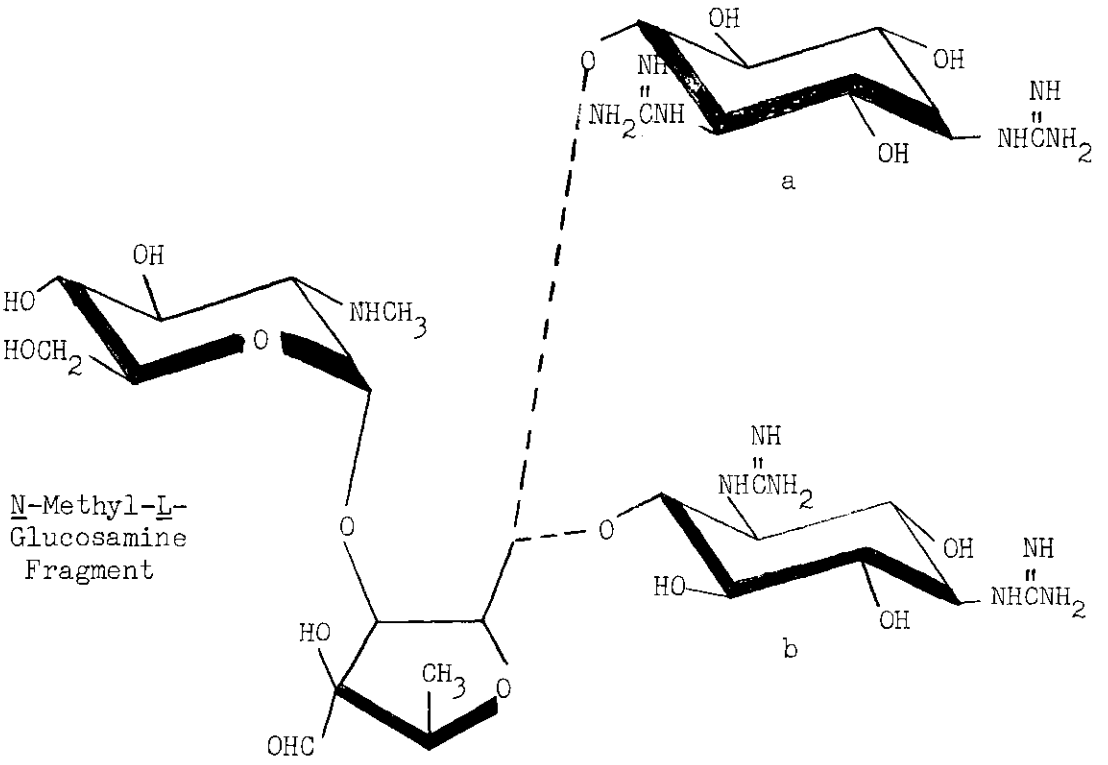
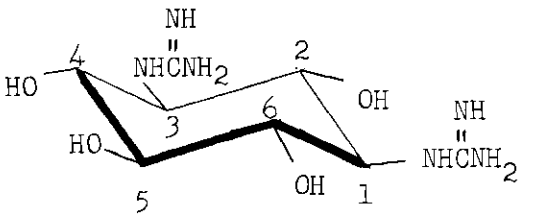
SUMMARY

At the start of this research the important antibiotic streptomycin was known to have either structure Ia or Ib, which differ only in the absolute configuration of the carbon atoms of the streptidine ring.



Streptomycin (I)

Streptidine has been shown to be the all equatorial isomer of 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane (XII). Streptidine thus possesses a plane of symmetry and is optically inactive. In the intact



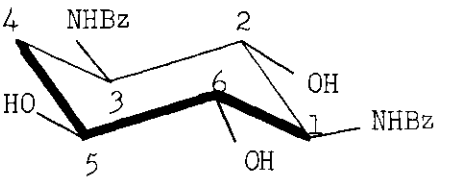
XII

streptomycin molecule, however, the streptobiosamine moiety is attached to the streptidine moiety through the hydroxyl group on C_4 of streptidine. This destroys the plane of symmetry of the streptidine portion of the molecule and causes all six of the carbon atoms of the streptidine ring to be asymmetric. In structure Ia the absolute configuration of the streptidine ring carbon atoms is 1(S), 2(S), 3(R), 4(S), 5(S), 6(R); in structure Ib, 1(R), 2(R), 3(S), 4(R), 5(R), 6(S).

Since the relative configurations of the carbon atoms of the streptidine ring in streptomycin are known to be all equatorial, the determination of the absolute stereochemistry about any one carbon atom would automatically determine the stereochemistry about the other five. A degradative procedure seemed desirable in order to obtain from the rather large streptomycin molecule a smaller fragment containing at least one of the streptidine carbon atoms whose original position in the ring was known and whose stereochemistry was known to be undisturbed by the degradative

sequence.

Since the preparation of N,N'-dibenzoyl-4-deoxystreptamine (XXI, or its mirror image) had been reported previously, it was thought that the possibility of degrading this compound to a substance of known



XXI

stereochemistry should be investigated. (The $-CH_2-$ of 4-deoxystreptamine contains the carbon atom to which the streptobiosamine fragment is attached in streptomycin.) The compound was prepared by use of the reaction sequence used by the original workers. Attempts to degrade XXI to aspartic acid by the use of nitric acid were unsuccessful. Degradation of XXI to N-benzoylaspartic acid was unsuccessfully attempted using permanganate ion under varying conditions of temperature, reaction time, and pH. A proposed five-step degradation of XXI to D- or L-serine using periodate oxidation failed in the second step. A six-step degradation of XXI to D- or L-2-aminopropan-1-ol failed in the third step. N,N'-Dibenzoylstreptamine was used as a model compound throughout the degradation attempts.

Reeves has developed a powerful method for the determination of the absolute configuration of many glycols. The method involves the measurement of the shift in optical rotation when an optically active glycol is

complexed with "cuprammonium" - a solution of ammonium hydroxide and copper(II) ion. If the projected angle formed by the two hydroxyl groups involved is positive, the rotational shift* observed upon complex formation will be positive; and if negative, the shift will be negative. This method has recently been successfully applied to the determination of the absolute configuration of the 2-deoxystreptamine fragment of the neomycins, kanamycins, and paromomycins.

Reeves' method could not be successfully applied to the determination of the absolute configuration of C_5 and C_6 in N,N'-dibenzoyl-4-deoxystreptamine because of the limited solubility of this compound in water. Conversion of the dibenzoyl compound to the corresponding diacetyl compound eliminated solubility difficulties.

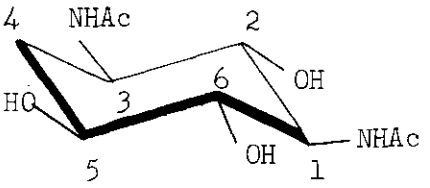
The method was tested on methyl α-D-mannopyranoside, a compound for which the rotational shift in Cupra B (a standard cuprammonium solution) was known. Results showed that the technique and apparatus used were certainly adequate.

The procedure was then applied to N,N'-diacetyl-4-deoxystreptamine (XLVI or XLVII). The specific rotation at 436 mμ of a water solution of the compound was found to be $+5 \pm 9^0$. The average of six determinations of the specific rotation at 436 mμ of Cupra B solutions of the compound was -970 ± 60^0. There was no doubt that the rotational shift was negative. The value calculated was -2400 ± 170^0. Since the rotational shift was negative, the projected angle formed by the hydroxyls on C_5 and C_6 of

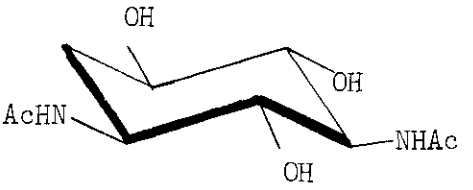
---

* $\Delta[M]_{\text{Cupra B}} = ([\alpha]_{436,\ \text{Cupra B}} - [\alpha]_{436,\ \text{water}})$ Mol. Wt./100

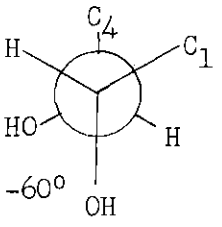
the diacetyl compound must be clockwise -60°. The compound thus has the absolute stereochemistry shown in structure XLVI and not that in structure XLVII.

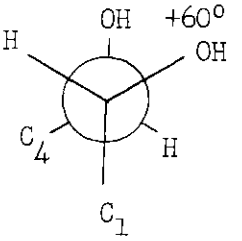


XLVI



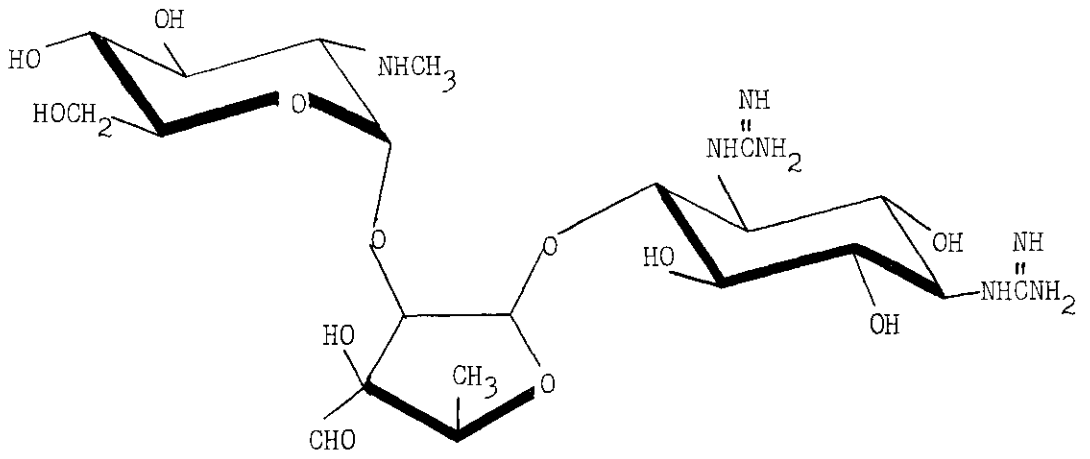
XLVII





Therefore, streptomycin has structure Ib (and not Ia) in which the absolute configuration of the carbon atoms of the streptidine ring is 1(R), 2(R), 3(S), 4(R), 5(R), 6(S).

This stereochemical result also establishes the complete structure determination of dihydrostreptomycin, hydroxystreptomycin, and mannosidostreptomycin. The configurational determination of the asymmetry of the streptidine ring in streptomycin yields the same result (i.e., R at C_4)



Ib

Streptomycin

as that determined for the 2-deoxystreptamine ring of the neomycins, kanamycins, and paromomycins. Thus these two vital components of a number of powerful antibiotics may have stereochemically similar biogenetic precursors.

CHAPTER I

INTRODUCTION

Isolation, Use, and Properties of Streptomycin

Waksman, Schatz, and Bugie in 1944 obtained a crude concentrate of an antibiotic substance from cultures of Streptomyces grisseus originally isolated from the soil (1).

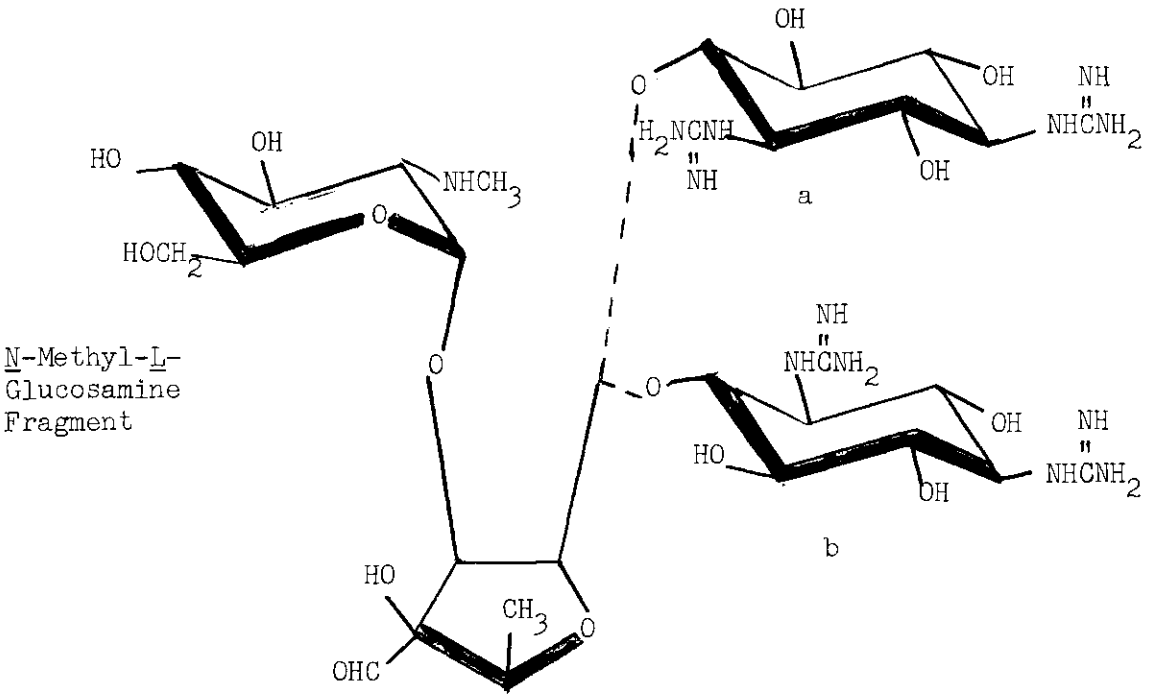
This substance, streptomycin (Ia or Ib), was found to be active against many gram-negative, gram-positive, acid-fast, and spirochaetal species of bacteria but inactive against anaerobic bacteria, protozoa, viruses, and the majority of fungi (2). It proved to be the first drug effective against tuberculosis (2), and the most important application of streptomycin and its close relative, dihydrostreptomycin, has been in the treatment of that disease. The streptomycin family of antibiotics probably is second in importance only to the penicillins.

Streptomycin was found to be a water-soluble, thermostable, acid- and alkali-labile, levorotatory, basic compound (3). By means of molecular weight determinations and elemental analyses of several crystalline salts, the molecular formula $C_{21}H_{37-39}N_7O_{12}$ was suggested for the antibiotic (3). Later investigations established the formula $C_{21}H_{39}N_7O_{12}$ for streptomycin.

Structure of Streptomycin

Structure of Streptobiosamine

Treatment of streptomycin with methanol containing hydrogen

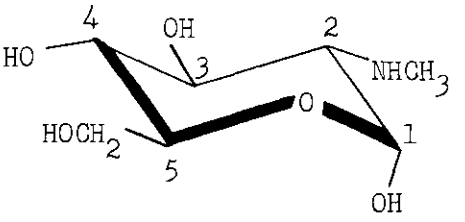


Streptomycin (I)

chloride gave a mixture of two products separable by chromatography into the dihydrochloride salt of a strong base named streptidine ($C_8H_{18}N_6O_4 \cdot 2HCl$) and into a compound later identified as methyl streptobiosaminide hydrochloride dimethyl acetal. Analytical and molecular weight data on the tetraacetyl derivative of the latter compound suggested the molecular formula $C_{13}H_{23}NO_9$ for the parent compound, streptobiosamine (3).

The rich oxygen content of streptobiosamine suggested that it might

be a disaccharide. Acid hydrolysis of methyl streptobiosaminide dimethyl acetal followed by acetylation yielded a pentaacetyl derivative of a hexosamine whose structure was deduced by characterization and confirmed by synthesis to be N-methyl-L-glucosamine (II) (4). The hexosamine was shown to exist in the pyranose ring form in streptobiosamine by means of periodate oxidation of N-acetyldeoxystreptobiosamine (5).



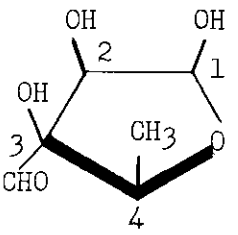
II

L-Streptose, the remaining hexose-like portion of streptobiosamine, was deduced to have structure III on the basis of chemical and physical properties of derivatives and degradation products and of various reactions of streptobiosamine (6). The stereochemistry of streptose has been fully elucidated (6). It was shown that C_2 of L-streptose and C_1 of N-methyl-L-glucosamine were joined by a glycosidic linkage having the α-L configuration (6,7). Thus, streptobiosamine has the structure and configuration shown in IV.

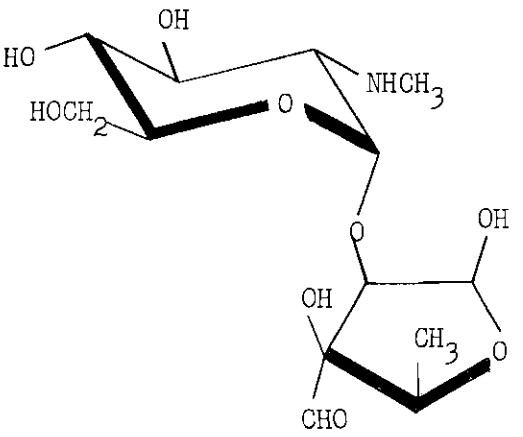
Structure of Streptidine

Streptidine was found to be optically inactive and to possess the molecular formula $C_8H_{18}N_6O_4$. It contained two guanido groups that accounted for all six nitrogen atoms and its strongly basic character, and four

acetylatable hydroxyl groups that accounted for all the oxygen atoms (8,9,10,11).



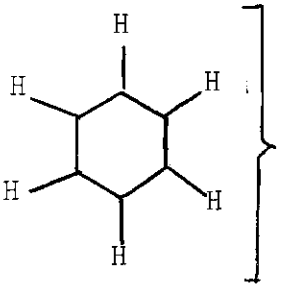
III

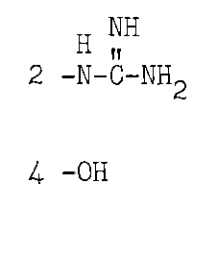


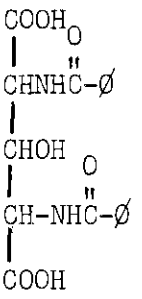
IV

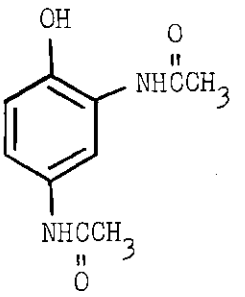
By boiling an aqueous barium hydroxide solution of streptidine under reflux for 24 hr., a new base, streptamine ($C_6H_{14}N_2O_4$), was obtained. These conditions were shown to have caused the degradation of the two guanido groups to two amino groups; two moles of carbon dioxide and four moles of ammonia were formed per mole of streptidine (9,12). The consumption of six moles of periodic acid by streptamine, without the liberation of formaldehyde, showed the absence of primary alcohol groups. That observation, along with the molecular formula of streptidine and the chemically and physically proved absence of carbon-carbon unsaturation, suggested a six-membered, carbocyclic structure (V) in which the two guanido groups were in the 1,2-, 1,3-, or 1,4- positions with hydroxyl

groups located on the other four carbon atoms (9,11,12).





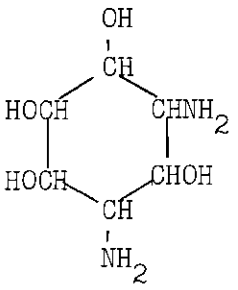




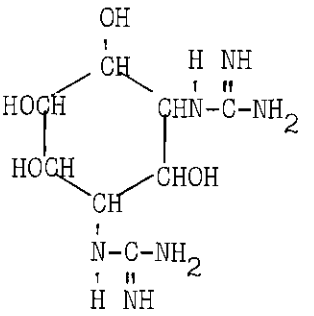
V VI VII

Hexaacetylstreptamine and hexabenzoylstreptamine were obtained as crystalline derivatives; from these compounds N,N'-diacetylstreptamine and N,N'-dibenzoylstreptamine were prepared by partial deacylation (9,10). Oxidation of N,N'-dibenzoylstreptamine with periodate yielded a crystalline five-carbon dialdehyde which upon oxidation with bromine water gave the dibenzamidohydroxyglutaric acid VI (13). That product would be expected only from 1,3-dibenzamido-2,4,5,6-tetrahydroxycyclohexane and not from the 1,2- or 1,4-dibenzamido isomer. Further support for the 1,3-diguanido assignment in streptidine was found in the observation of pyrolytic aromatization of hexaacetylstreptamine which gave 2,4-diacetamidophenol (VII) (11). Therefore, streptamine was established as 1,3-diamino-2,4,5,6-tetrahydroxycyclohexane (VIII) and streptidine as the corresponding diguanido compound (IX) (11).

Since streptidine was optically inactive, eight meso forms were possible for its configuration. The structure of streptidine was confirmed by synthesis from D-glucosamine (X) through compound XI (14). This

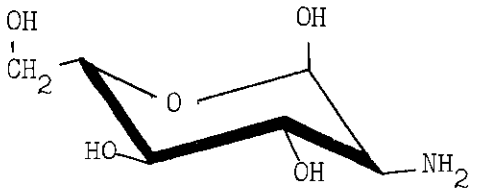


VIII

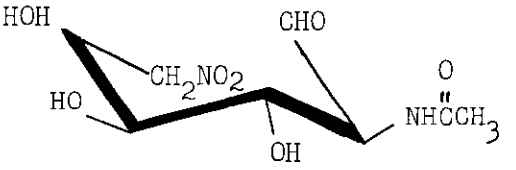


IX

synthesis established that the groups on C_1, C_5, and C_6 were configurationally all equatorial to each other.



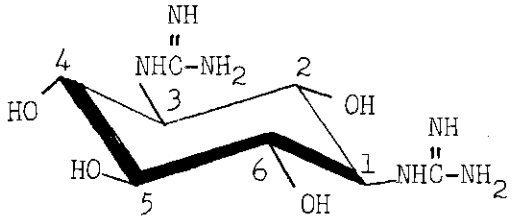
X



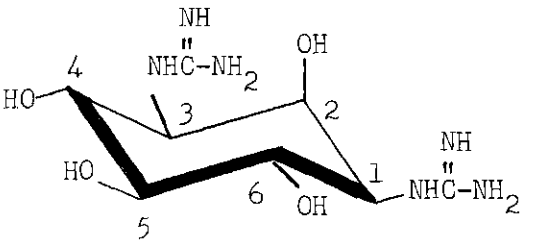
XI

Of the eight theoretically possible isomers obtainable from alkaline condensation of XI and subsequent transformation to streptidine, six would be optically active and two would be meso forms. Since streptidine was optically inactive, only the meso structures XII and XIII (or their mirror image) which differ in the configuration of C_2, were possible structures for streptidine. Fischer, Grosheintz, and Baer have found

considerable evidence that alkali-catalyzed carbonyl condensations of the type employed to join C_2 and C_3 lead only to <u>trans</u> configurations when used on optically active compounds (15,16,17). This would tend to eliminate structure XIII, since in that structure the groups on C_2 and C_3 have a <u>cis</u> configuration. Very good support for the assignment of structure XII to streptidine was provided by the work of Wintersteiner and Klingsberg (18). These workers prepared compound XIV from streptamine. When XIV was



XII

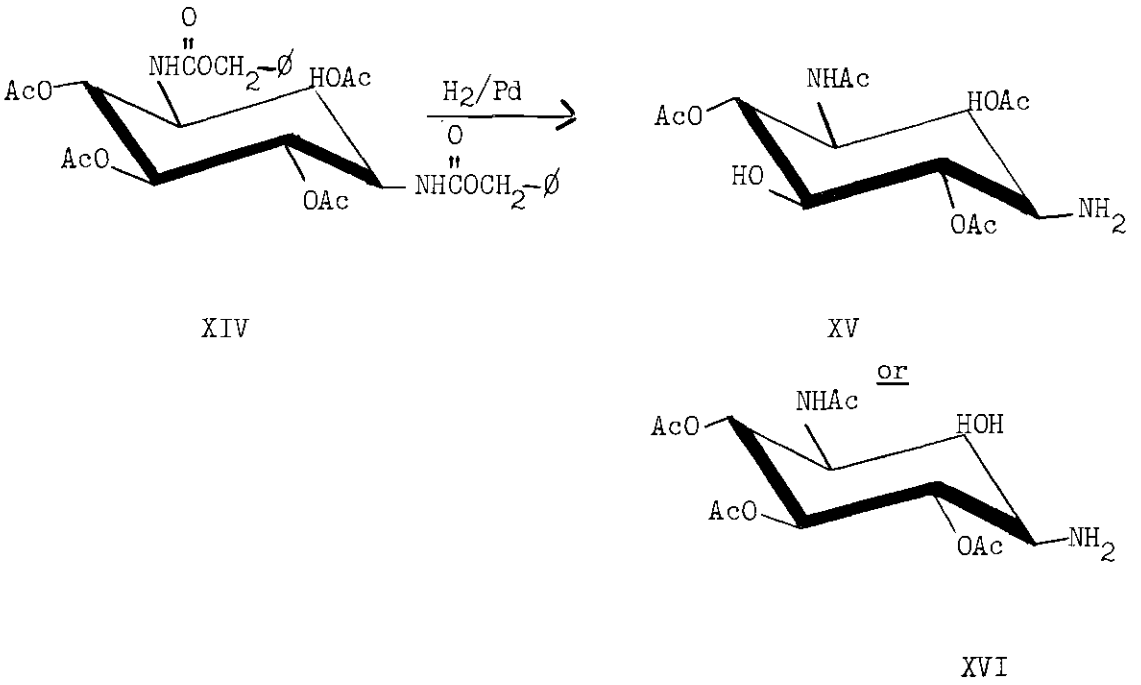


XIII

reduced with hydrogen and palladium, the product found was one in which acetyl migration from a hydroxyl to an amino group had occurred. It is well established that acetyl migrations of this type occur much more readily between <u>cis</u> hydroxyl and amino groups than between <u>trans</u> groups. Thus, the two possible structures for the compound formed were XV and XVI. But the compound was inert to periodate oxidation; this ruled out structure XVI. Therefore, since acetyl migration occurred from C_5 and not C_2, the hydroxyl group on C_2 was in all probability <u>trans</u> to the amino groups on C_1 and C_3 and streptidine would have structure XII and not structure XIII.

<u>Union of Streptobiosamine and Streptidine</u>

As shown in the accompanying equation, anhydrous methanol that



XIV

XV

XVI

contained hydrogen chloride cleaved streptomycin, with the consumption of three moles of methanol, into streptidine dihydrochloride and methyl streptobiosaminide hydrochloride dimethyl acetal (19).

$$C_{21}H_{39}N_7O_{12}\cdot 3HCl + 3CH_3OH \rightarrow C_8H_{18}N_6O_4\cdot 2HCl + H_2O + C_{13}H_{22}NO_7(OCH_3)_3\cdot HCl$$

The aldehyde group in the streptose portion of streptomycin was converted into the dimethyl acetal; the third methoxy group consumed was suspected to have formed a methyl glycoside. The suspected presence of a glycosidic linkage between the streptidine and streptobiosamine moieties was substantiated as follows.

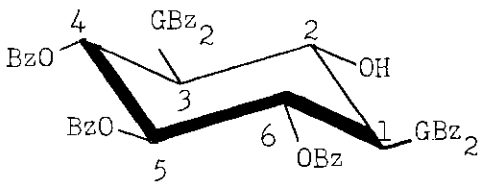
Dihydrostreptomycin trihydrochloride, in which the aldehyde group of the streptose fragment has been reduced to a primary alcohol group, was cleaved by methanolic hydrogen chloride, and the streptidine dihydrochloride

was removed by chromatography. Acetylation of the remaining amorphous product led to the isolation of two crystalline acetyl derivatives, m.p. 198-198.5° and m.p. 155.5-157°, both of which had the molecular formula $C_{24}H_{37}NO_{14}$. Both compounds contained one methoxy group and five acetyl groups, four of which were attached to oxygen atoms and one to the nitrogen atom. In order to investigate the nature of the isomerism involved in these two compounds, they were separately treated with ethyl mercaptan and hydrogen chloride to replace the methoxy group by an ethylmercapto group. After reacetylation, they were treated with Raney nickel and hydrogen to substitute a hydrogen atom for the ethylmercapto group. Both isomers gave the same crystalline product, pentaacetyldihydrodeoxystreptobiosamine. From that behavior, the isomerism was in all probability due to anomeric differences about a carbon atom bearing a glycosidic methoxy group. From the known structure of streptobiosamine (IV), the hemiacetal hydroxyl group of the streptose portion of streptobiosamine was almost certainly involved in the linkage between streptobiosamine and streptidine.

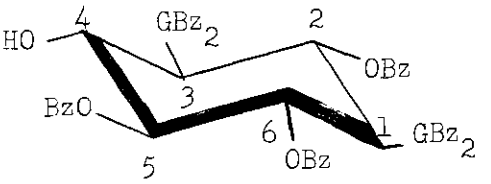
By use of Hudson's rules of isorotation (20), the anomeric structure of the streptobiosamine-streptidine linkage in streptomycin was calculated to be β-L as shown in structure Ia or Ib (7).

Upon treatment with benzoyl chloride and pyridine, streptomycin gave undecabenzoylstreptomycin, which was then degraded by hydrogen bromide in acetic acid to heptabenzoylstreptidine and tetrabenzoylstreptobiosamine (21). Because heptabenzoylstreptidine gave more than one equivalent of dibenzoylguanidine upon chromic acid oxidation, streptobiosamine must not be linked to streptidine through a nitrogen atom. Thus, since streptidine was known to have structure XII (14,18), the possible position

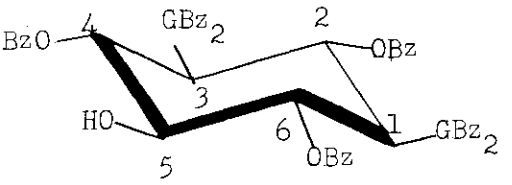
of attachment was through the oxygen atom on C_2 or C_5 or C_4 (C_4 and C_6 are equivalent except for absolute streochemistry). The corresponding structures possible for heptabenzoylstreptidine were XVII, XVIII, or XIX (or their mirror images). Because heptabenzoylstreptidine was op-



XVII



XIX



XVIII

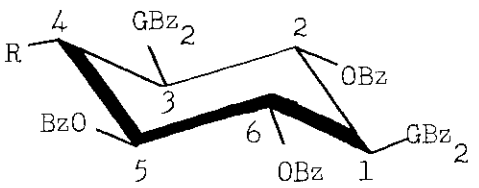
Where: Bz = $C_6H_5\overset{\overset{O}{\|}}{C}-$

G = $-\overset{|}{N}-\overset{\overset{NH}{\|}}{C}-NH-$

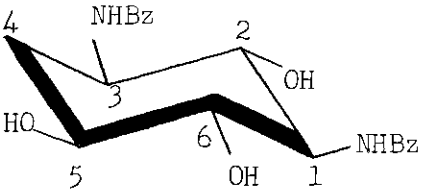
tically active (21), the unbenzoylated hydroxyl could not be either at C_2 or C_5 since these positions are in a plane of symmetry and would result in optically inactive heptabenzoylstreptidine. Therefore, on stereochemical grounds, heptabenzoylstreptidine must possess the optically active structure XIX (or its mirror image) in which the original position of attachment of streptobiosamine was through the oxygen atom on C_4.

Further evidence for attachment at C_4 of streptidine was found by

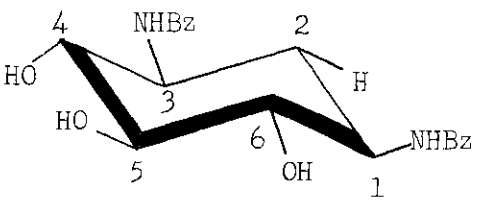
a series of degradative reactions (22). Heptabenzoylstreptidine was reacted with methanesulfonyl chloride; this gave heptabenzoyl-4-O-mesyl-streptidine (XXa) which, when treated with sodium iodide in acetone at 100°, gave heptabenzoyl-4-iodostreptidine (XXb) (the formation of this iodo compound constitutes further evidence for an oxygen atom rather than a nitrogen atom linking streptidine and streptobiosamine). Heptabenzoyl-4-iodostreptidine underwent hydrogenolysis over Raney nickel



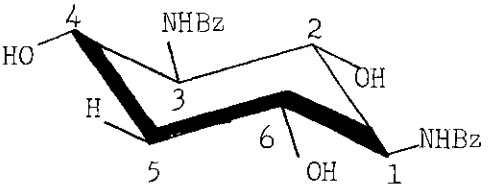
XX



XXI



XXII



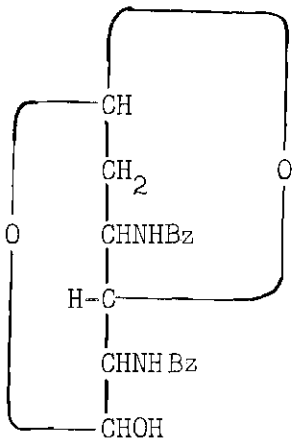
XXIII

Where: G = $-\overset{\text{NH}}{\overset{\|}{\text{N}}}-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{NH}$, Bz = $C_6H_5\overset{\text{O}}{\overset{\|}{\text{C}}}-$

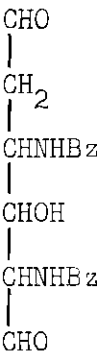
R = $-OSO_2CH_3$ (a) , -I (b) , -H (c)

catalyst to heptabenzoyl-4-deoxystreptidine (XXc), which was debenzoylated in methanol solution with barium methoxide. The resulting 4-deoxystreptidine was then hydrolyzed to 4-deoxystreptamine by the action of boiling aqueous barium hydroxide. 4-Deoxystreptamine was benzoylated; this gave pentabenzoyl-4-deoxystreptamine, which was selectively debenzoylated in methanol solution with barium hydroxide to N,N'-dibenzoyl-4-deoxystreptamine (XXI). N,N'-Dibenzoyl-4-deoxystreptamine consumed only one mole of periodate in agreement with structure XXI (no periodate would have been consumed by a compound of structure XXIII, and two moles would have been consumed were structure XXII the correct one for N,N'-dibenzoyl-4-deoxystreptamine).

The product from the periodate reaction was shown by chemical and physical evidence to have structure XXIV (or its mirror image), a cyclized form of the expected dialdehyde (XXV) (22). Thus, the evidence for attachment of streptobiosamine to streptidine through the oxygen atom at C_4 appears conclusive.



XXIV



XXV

Statement of the Problem

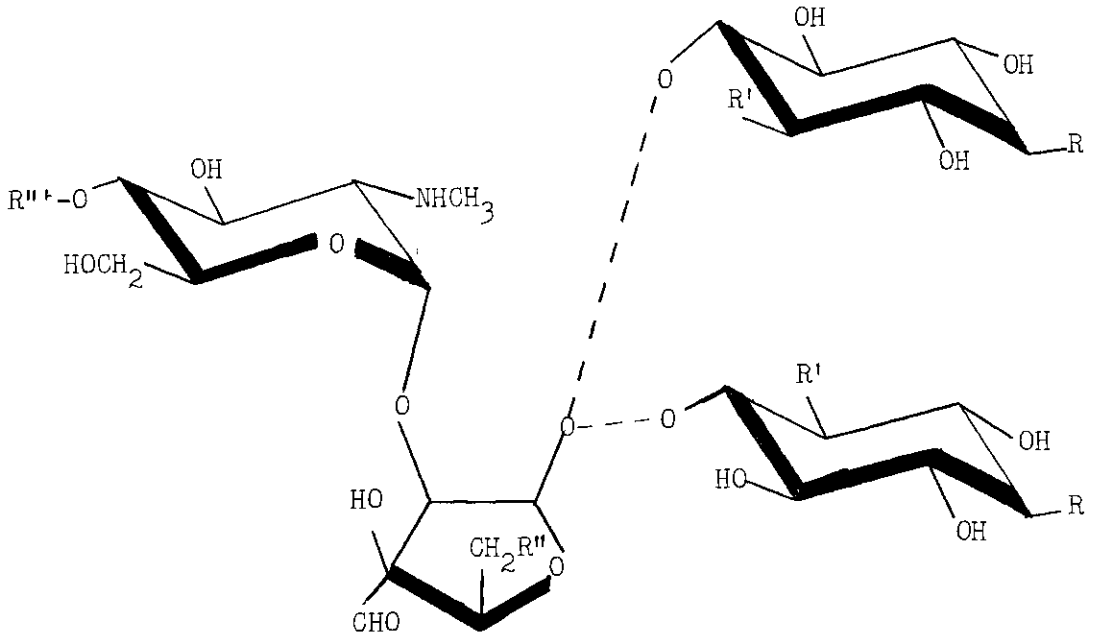
The evidence thus far accumulated shows that streptomycin has either structure Ia or structure Ib, which differ only in the absolute stereochemistry of the carbon atoms of the streptidine ring in the intact streptomycin molecule. In structure Ia the carbon atoms of the streptidine ring have the absolute configuration 1(S), 2(S), 3(R), 4(S), 5(S), 6(R), while in structure Ib the configuration is 1(R), 2(R), 3(S), 4(R), 5(R), 6(S). (This very fine method of denoting absolute configuration was developed by Cahn, Ingold, and Prelog (23)).

Tatsuoka and Horii (24) have suggested that the streptidine carbon atoms have the latter configuration based on the fact that N,N'-diacetyl-2,5,6-tri-O-methylstreptamine, derived from dihydrostreptomycin, has the same sign (positive) of rotation as N,N'-diacetyl-5,6-di-O-methyl-2-deoxystreptamine, derived from pseudoneamine (25).

The purpose of the present research was to confirm whether Ia or Ib was the correct structure for streptomycin by determination of the absolute configuration of the carbon atoms of the streptidine ring.

Significance of the Problem

This research proposes to complete the stereochemical formula of streptomycin, a natural product of more than routine interest. A rather large volume of reports have dealt with various aspects of the biosynthesis and mode of action of streptomycin (26,27,28,29), but little has been conclusively established. A complete account of neither the biosynthetic pathway nor the mode of action of streptomycin can result until the complete structure is known. The present research should enable more meaningful results to be obtained in these areas of research.

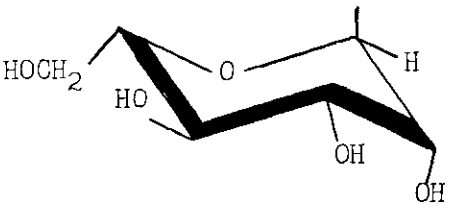


XXVI

Where: XXVIa. R = R' = $-NH-\overset{\overset{NH}{\|}}{C}-NH_2$, R" = -OH, R"' = H

(Hydroxystreptomycin)

XXVIb. R = R' = $-NH-\overset{\overset{NH}{\|}}{C}-NH_2$,



R" = H, R"' =

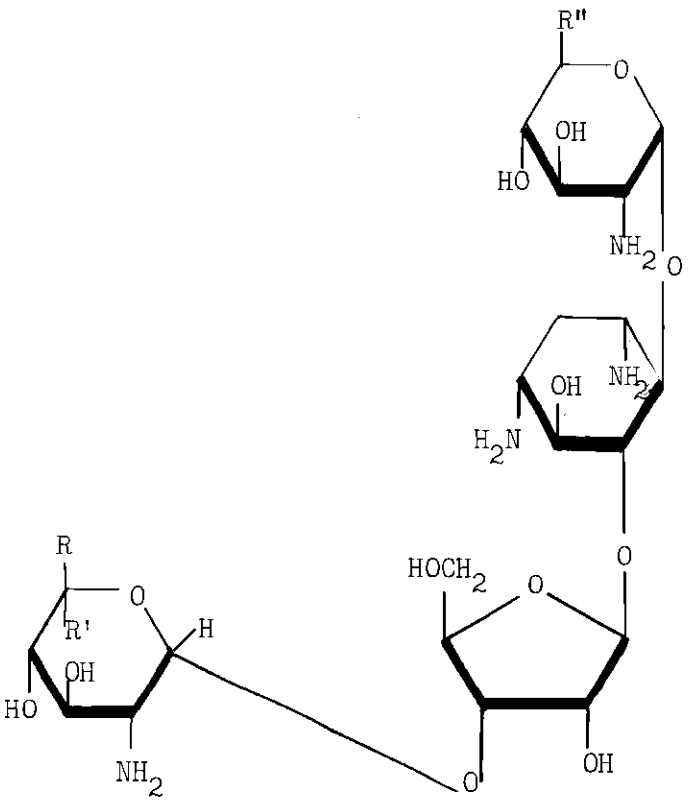
(Mannosidostreptomycin)

XXVIc. R = $-NH-\overset{\overset{NH}{\|}}{C}-NH_2$, R' = $-O-\overset{\overset{O}{\|}}{C}-NH_2$, R" = R"' = H

<u>or</u> R = $-O-\overset{\overset{O}{\|}}{C}-NH_2$, R' = $-NH-\overset{\overset{NH}{\|}}{C}-NH_2$, R" = R"' = H

(Bluensomycin)

Furthermore, streptidine, streptamine, and deoxystreptamine moieties occur not infrequently in antibiotics. The naturally occurring antibiotics dihydrostreptomycin (aldehyde group of the streptose fragment of streptomycin reduced to a primary alcohol group), hydroxystreptomycin ($C\text{-}CH_3$ group of the streptose fragment of streptomycin changed



XXVII

Where: XXVIIa, R = H, R' = CH_2NH_2, R" = CH_2NH_2 (suggested stereochemistry).

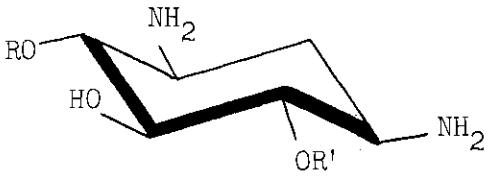
XXVIIb, R = R" = CH_2NH_2, R' = H

XXVIIc, R = H, R = CH_2NH_2, R" = CH_2OH (suggested stereochemistry).

XXVIId, R = CH_2NH_2, R' = H, R" = CH_2OH (suggested formula).

to a hydroxymethyl group) (XXVIa)(30),and mannosidostreptomycin (XXVIb) (31) contain the streptidine moiety; and bluensomycin (XXVIc)(32) contains a streptidine-like moiety.

2-Deoxystreptamine is a component of neomycin B (XXVIIa)(33,25), neomycin C (XXVIIb)(31,23), paromomycin I and II (XXVIIc and XXVIId) (34,25), and the kanamycins (XXVIII)(35).



XXVIII

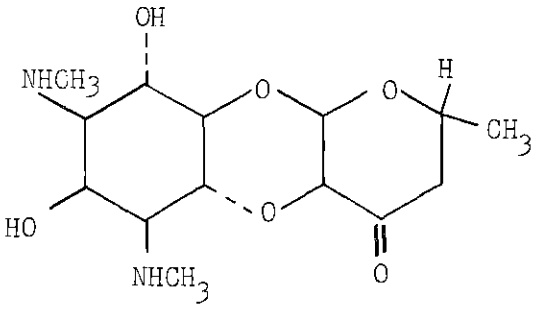
Where: R = D-Glucosamine, R' = 3-amino-3-deoxy-D-glucose (Kanamycin C)

R = 6-Aminoglucose, R' = 3-amino-3-deoxy-D-glucose (Kanamycin A)

R = Diaminohexose, R' = 3-amino-3-deoxy-D-glucose (Kanamycin B)

Actinospectin (XXIX) (36) contains an isomer of N,N'-dimethylstreptamine. The relative stereochemistry of the actinamine portion of actinomycin has been shown (36) to be as indicated, and not all equatorial.

The present research thus may prove useful in the study of the chemistry of other antibiotics as well as that of streptomycin.

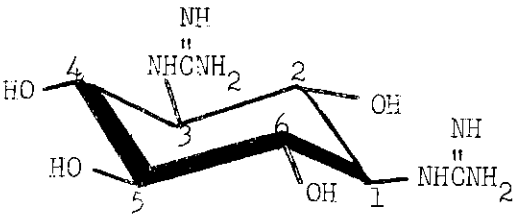


XXIX

CHAPTER II

DISCUSSION OF RESULTS

Streptidine has been shown to be the all equatorial isomer of 1,3-diguanido-2,4,5,6-tetrahydroxycyclohexane (XII) (11,14,18). Streptidine thus possesses a plane of symmetry and is optically inactive. In the intact streptomycin molecule (Ia or Ib), however, the streptobiosamine moiety is attached to the streptidine moiety through the hydroxyl group on C_4 of streptidine. This destroys the plane of symmetry of the streptidine portion of the molecule and causes all six of the carbon atoms of the streptidine ring to be asymmetric. Since the relative configurations of the carbon atoms of the streptidine ring in streptomycin are known to be all equatorial, the determination of the absolute stereochemistry about any one carbon atom would automatically determine the stereochemistry about the other five.



XII

A degradative procedure seemed desirable in order to obtain from the rather large streptomycin molecule a smaller fragment containing at

least one of the streptidine carbon atoms whose original position in the ring was known and whose stereochemistry was known to be undisturbed by the degradative sequence.

The preparation of N,N'-dibenzoyl-4-deoxystreptamine (XXI, or its mirror image) from streptomycin or dihydrostreptomycin has been reported by Kuehl, Peck, Hoffhine, Peel, and Folkers (21,22). It was thought that it might be possible to degrade this compound to a substance that was of known stereochemistry. The reaction sequence used by the original workers was followed for the preparation of the compound.

Benzoylation of dihydrostreptomycin by use of benzoyl chloride and pyridine gave a 106% yield of crude polybenzoyldihydrostreptomycin. Contaminants were doubtless the cause of the higher-than-theoretical yield. A melting point of 137-141° was found for the crude material and 141-145° for the once-reprecipitated, crude polybenzoyldihydrostreptomycin. No melting point was reported in the literature. A value of +85° was found for the optical rotation of the once-reprecipitated material. The original workers reported +51° for the optical rotation of pure dodecabenzoyldihydrostreptomycin isolated in 4% yield from the crude polybenzoyl material obtained in 79% yield. An infrared spectrum of a 1% solution of our crude material in chloroform showed peaks at 5.80 and 5.95 μ, among others. These were assigned to the ester and guanidineamide carbonyl groups, respectively. Elemental analyses indicated that the crude material averaged 10-11 benzoyl groups per molecule. It would have been possible to purify the crude material by chromatography (as done by the original workers), but the loss of time and material which would certainly have ensued made it undesirable unless necessary. Any of the polybenzoyl

material which had a fully benzoylated streptidine portion would yield the next product in the sequence.

The crude polybenzoyldihydrostreptomycin was cleaved by the action of hydrogen bromide in acetic acid to heptabenzoylstreptidine (XIX, or its mirror image) and methyl streptobiosaminide dimethyl acetal, which were separated by exploiting their great difference in solubility in methanol-benzene. Heptabenzoylstreptidine of high purity was obtained in rather good yield (usually about 70%), thus justifying the use of crude polybenzoyldihydrostreptomycin. The original workers reported a yield of 54%. The melting point was 250-252°, six degrees lower than that reported in the literature. Two recrystallizations caused a sharpening to 251-252° but no rise in the melting point. The substance had an optical rotation of +53°, which is in good agreement with the +54° reported for the compound. An infrared spectrum of a 1% solution of the compound in chloroform showed peaks at 5.80 and 5.96 μ, among others. These were assigned to the ester and guanidine-amide carbonyl groups, respectively. Analytical results agreed with those calculated for heptabenzoylstreptidine.

Heptabenzoyl-4-O-mesylstreptidine (XXa) was easily prepared by the action of methanesulfonyl chloride on a cold pyridine solution of heptabenzoylstreptidine. A yield of about 85% was usual; the original workers reported a 91% yield. Both melting point and optical rotation agreed with that reported for the compound, so analytical data were deemed unnecessary. An infrared spectrum of a 1% solution of the material in chloroform showed peaks at 5.80, 5.96, and 8.54 μ, among others. The peaks at 5.80 and 5.96 μ were assigned to the ester and guanidine-amide

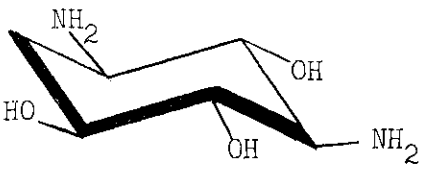
carbonyl groups, respectively. The peak at 8.54 μ was assigned to the mesyl group by observation of the same peak in mesylcholesterol and its absence in cholesterol itself. This peak was absent in the spectra of heptabenzoylstreptidine and heptabenzoyl-4-iodostreptidine. An n.m.r. spectrum of the mesyl compound showed the protons of the methanesulfonyl group as a sharp singlet at 7.09 τ. A sharp singlet at 6.99 τ due to the methanesulfonyl group was present in the spectrum of mesylcholesterol.

A mixture of heptabenzoyl-4-O-mesylstreptidine, sodium iodide, and acetone was heated in a sealed bottle at steam bath temperature for four hours. From the reaction mixture crude heptabenzoyl-4-iodostreptidine (XXb) was isolated in yields of about 110% based on pure material. This higher-than-theoretical yield was due in all probability to contaminants present along with the desired iodo compound. The crude material had m.p. 143-145° and an optical rotation of +21° as compared to the reported values of m.p. 153-154° and rotation of +23° for the pure material. The crude iodo compound was purified by chromatography over silicic acid. Only about 50-60% of the theoretical amount of iodo compound produced in the reaction was recovered from the column, which suggested that the material originally isolated in the reaction was very impure. The original workers reported a 100% yield of crude material and an 87% yield of purified compound. The iodo compound had, after chromatography, m.p. about 146°. One recrystallization and thorough drying raised the melting point to 154-155°. The optical rotation of the recrystallized compound was +15.8°. Analytical data for the recrystallized material agreed with those calculated for heptabenzoyl-4-iodostreptidine. The infrared spectrum of a 1% solution of the compound in chloroform showed peaks at 5.77 and 5.95 μ,

among others. These were assigned to the ester and guanidine-amide carbonyl groups, respectively.

Hydrogenolysis of the iodo compound to heptabenzoyl-4-deoxystreptidine (XXc) was accomplished in a Parr pressure reaction apparatus under 40 p.s.i. of hydrogen. Ninety per cent dioxane-water was used as solvent and Raney nickel was used as catalyst. The product was purified by several recrystallizations. The usual yield of pure heptabenzoyl-4-deoxystreptidine was around 70%. The original workers obtained a 90% yield of crude material; no yield was reported for the purified compound. The pure compound had m.p. 198-199°, as reported in the literature, and an optical rotation of +47° in chloroform (no value for the rotation was reported). An infrared spectrum of a 1% solution of the compound in chloroform showed peaks at 5.78 and 5.94 μ, among others, assigned to the ester and guanidine-amide carbonyl groups. Analytical data for the compound agreed with that calculated for heptabenzoyl-4-deoxystreptidine.

Heptabenzoyl-4-deoxystreptidine was hydrolyzed to 4-deoxystreptidine by stirring for 22 hr. at room temperature a mixture of the compound, dry methanol, and dry barium hydroxide. The 4-deoxystreptidine was converted without purification or characterization to 4-deoxystreptamine (XXX) by the action of boiling aqueous barium hydroxide. This step



XXX

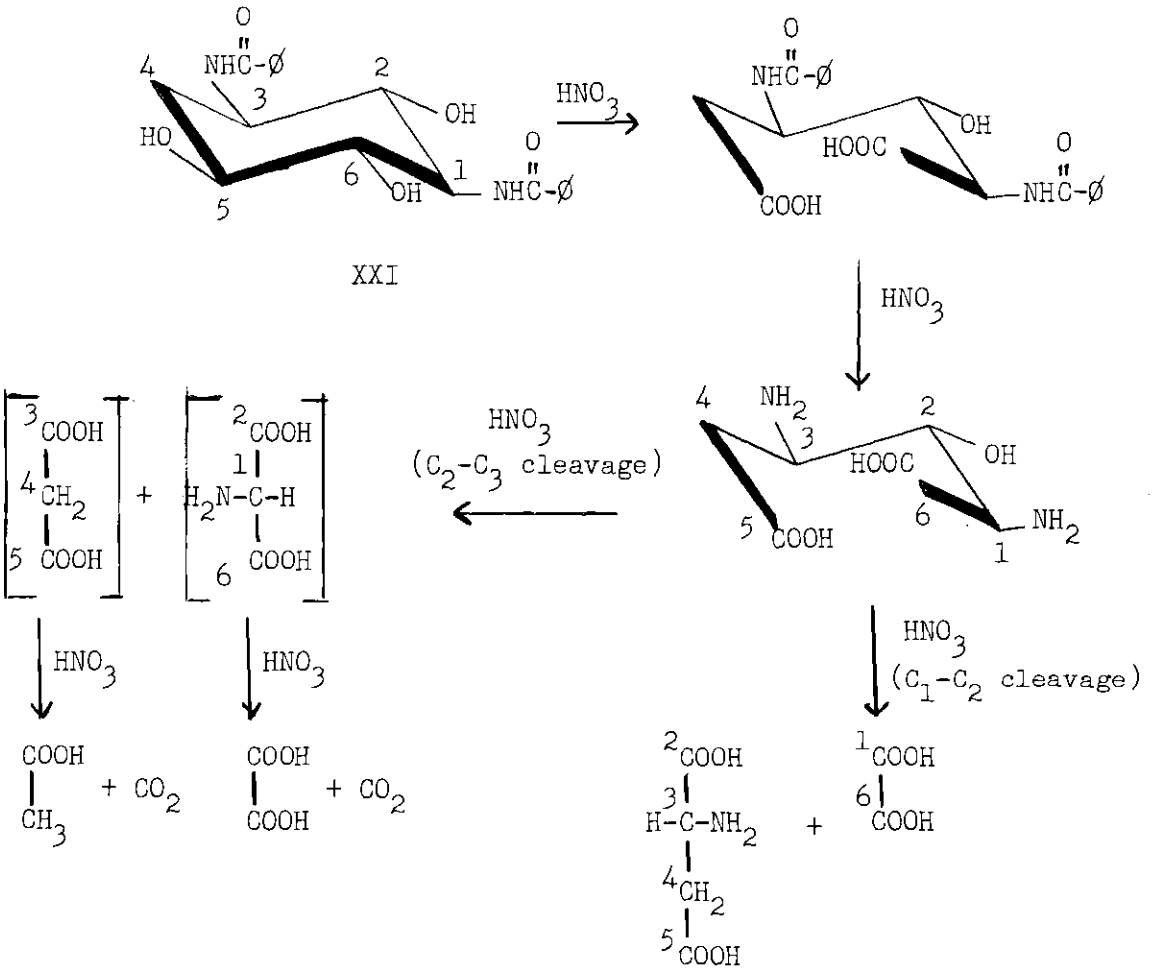
consisted of hydrolysis of the two guanido groups of 4-deoxystreptidine to amino groups.

The 4-deoxystreptamine was not purified or characterized but was converted to pentabenzoyl-4-deoxystreptamine by the action of pyridine and benzoyl chloride. The very crude pentabenzoyl material (m.p. 250-280° with decomp., reported m.p. 293-294°) was not purified in order to avoid loss of any material. The yield of crude pentabenzoyl compound was usually about 70% overall from heptabenzoyl-4-deoxystreptidine. An infrared spectrum of a 1% solution of the crude material in chloroform showed peaks at 5.80 and 5.99 μ, among others. These peaks were assigned to the ester and amide carbonyl groups, respectively.

The crude pentabenzoyl-4-deoxystreptamine was converted to N,N'-dibenzoyl-4-deoxystreptamine (XXI) by the action of dry barium hydroxide in methanol at 5°. The conditions used were sufficient to hydrolyze the three ester groups but not the two amide groups. N,N'-Dibenzoyl-4-deoxystreptamine of good purity was obtained in a usual yield of about 35% after chromatography on an alumina column and one recrystallization. A yield of 47% was reported by the original workers. Analytical data for the compound agreed with that calculated for N,N'-dibenzoyl-4-deoxystreptamine. The pure compound had m.p. 284-286° as compared to 287-289° reported. No determination of optical activity was made on the compound. The infrared spectrum of a nujol mull of the compound showed a peak at 5.99 μ, among others. It was assigned to the amide carbonyl group. No other peak was present between 5 and 6 μ; this showed the absence of any ester groups.

Nitric acid was the reagent used in the first attempts to degrade

N,N'-dibenzoyl-4-deoxystreptamine to a compound of known stereochemistry. This reagent had proved very valuable in degradation of sugars (37,38,39, 40,41). It was thought that nitric acid would possibly lead to D-aspartic acid (XXXI) from one enantiomer of the deoxy compound (XXI) by the sequence



XXI

XXXI

Fig. 1. Theoretical Degradation of N,N'-Dibenzoyl-4-deoxystreptamine to D- or L-Aspartic Acid.

shown as Fig. 1. The mirror image of XXI would lead to L-aspartic acid, the mirror image of XXXI.

Before attempting to oxidize N,N'-dibenzoyl-4-deoxystreptamine to aspartic acid, it was considered best to determine what effect nitric acid would have on a very closely related compound, N,N'-dibenzoylstreptamine (XXXII). This compound was obtained by Shotten-Baumann benzoylation of crude streptamine sulfate followed by hydrolysis of the ester groups with 0.5N sodium hydroxide in methanol (42). The compound melted at a lower temperature (280-285°, dec.) than that reported (293-295°, dec.) (42). An infrared spectrum of the compound in nujol was taken. A broad peak at 2.9-3.0 μ was assigned to N-H and O-H stretching. No peak was present in the region from 5.0 μ to 6.0 μ, which showed the absence of ester groups. A strong peak at 6.08 μ was assigned to the amide carbonyl. The spectrum was very similar to that of N,N'-dibenzoyl-4-deoxystreptamine.

Conditions that would theoretically lead to aspartic acid from the deoxy compound by the route shown as Fig. 1 should lead to glycine (XXXIII) from the model compound, N,N'-dibenzoylstreptamine (XXXII) by the route shown as Fig. 2.

A solution of N,N'-dibenzoylstreptamine in acetic acid-concentrated nitric acid was heated at ca. 95° under reflux for eight hours. Work-up of the reaction mixture yielded 61% of benzoic acid; this showed that hydrolysis had occurred at least to that extent. Also obtained was a small amount of yellow gum that gave a positive ninhydrin test, which suggested that glycine might be present. A negative Nessler's test showed the absence of ammonia. A paper chromatogram of the gum indicated the presence of a small amount of glycine in the gum and the absence of streptamine.

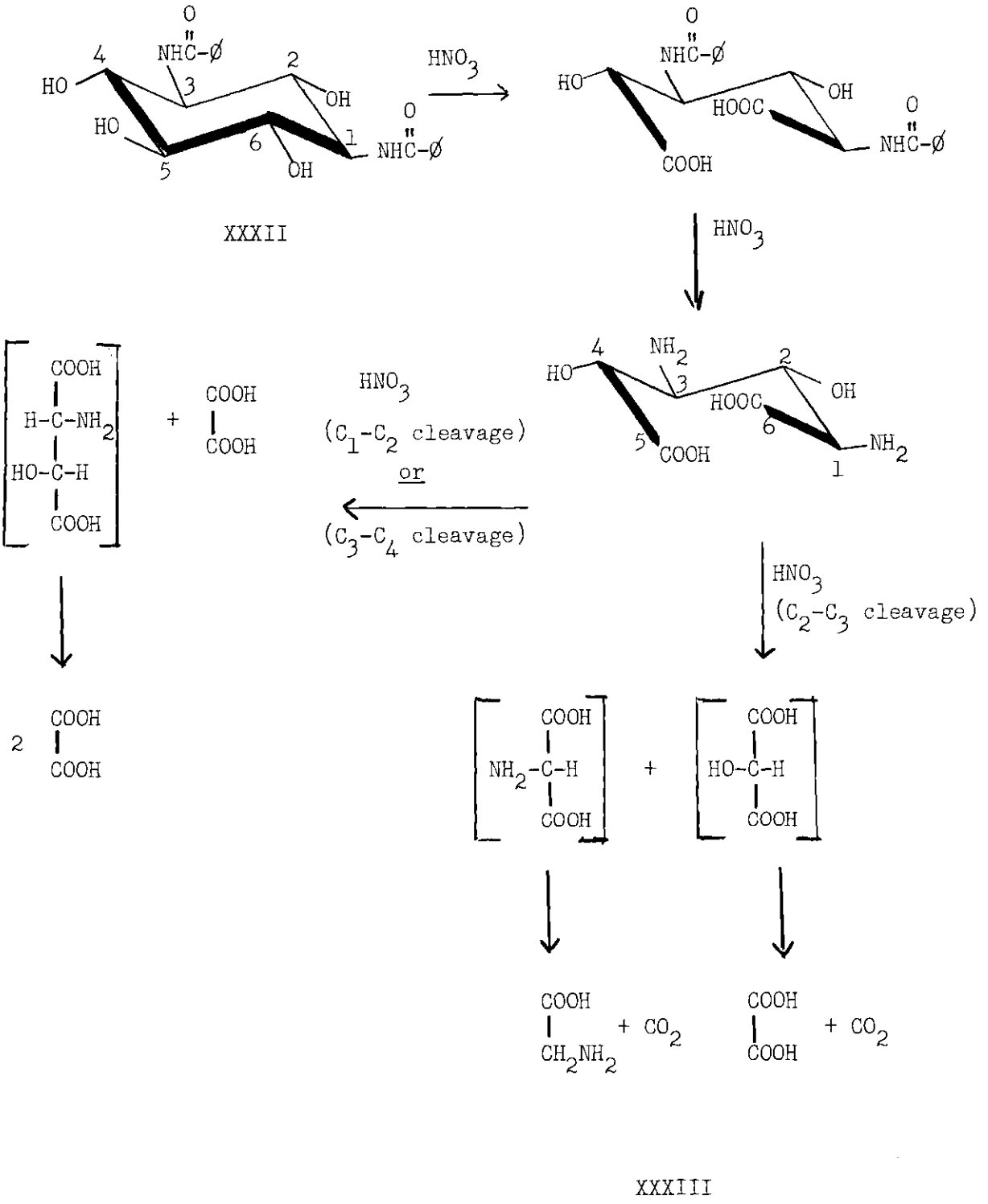


Fig. 2. Theoretical Degradation of N,N'-Dibenzoyl-streptamine to Glycine.

An oxidation carried out similarly except that a 46 hr. reaction time was used gave a gum that gave no spots on a paper chromatogram. When a reaction time of 3.5 hr. was used, the chromatogram of the gum obtained showed a multiplicity of spots. It thus seemed that a reaction time of around six hours might give best results.

Accordingly, an oxidation of the compound was carried out using a reaction time of six hours. Work-up of the reaction gave 83% of benzoic acid. The aqueous solution that remained was passed over an ion-exchange resin (IR-45, hydroxyl phase), and the column was washed with water. As the eluate came off the column it was tested with ninhydrin in order to ascertain when the glycine was coming off; however, no portion of the eluate gave a positive ninhydrin test. The total eluate was evaporated; this gave a small amount of gum that gave a negative ninhydrin test. More water was passed over the column. Upon evaporation of this eluate, a very small quantity of ninhydrin-positive gum was obtained. The column was then washed with 1N hydrochloric acid. Evaporation of the eluate gave a small amount of a ninhydrin-positive, Nessler-negative gum. A paper chromatogram of the two ninhydrin-positive products, alongside authentic glycine, indicated that the oxidation gave a complex mixture of products including, probably, a small quantity of glycine.

Although the results of nitric acid oxidation of the model compound were not too encouraging, one oxidation of the deoxy compound was attempted in hopes of finding aspartic acid. It was determined that aspartic acid was stable under the reaction conditions to be employed.

A solution of N,N'-dibenzoyl-4-deoxystreptamine in acetic acid-

concentrated nitric acid was heated at ca. 95° under reflux for six hours. From the reaction mixture was isolated 88% of benzoic acid. Also isolated was a small amount of gum, which gave a faint positive ninhydrin test and a negative Nessler's test. A paper chromatogram of the gum was run alongside authentic samples of glycine and aspartic acid. Only a faint, blue spot that did not correspond either to glycine or aspartic acid was obtained from the gum.

Permanganate oxidation was the next procedure tried. It was theorized that this reagent would attack the deoxy compound as shown in Fig. 3 and lead to either N-benzoyl-D-aspartic acid (XXXIV) from the enantiomorph shown (XXI) or to N-benzoyl-L-aspartic acid from its mirror image.

Trial oxidations were performed on the model compound, N,N'-dibenzoylstreptamine. This compound would be expected to react according to the sequence shown as Fig. 4 and yield hippuric acid, N-benzoylglycine (XXXV).

Accordingly, a solution of N,N'-dibenzoylstreptamine, water, and silver permanganate was boiled under reflux for eight hours. Work-up resulted in the recovery of 10% of the starting material and 36% of crude benzamide. Also obtained, from extracts that should have contained any acidic material present, was a small amount of slightly viscous, yellow liquid. The liquid was only very slightly soluble in boiling water. A very small quantity of white solid precipitated from the hot mixture when it cooled. The solid had m.p. about 70° and was probably benzoic acid. The yellow liquid was not further characterized.

Two oxidations at room temperature using potassium permanganate were tried. A solution of N,N'-dibenzoylstreptamine, potassium

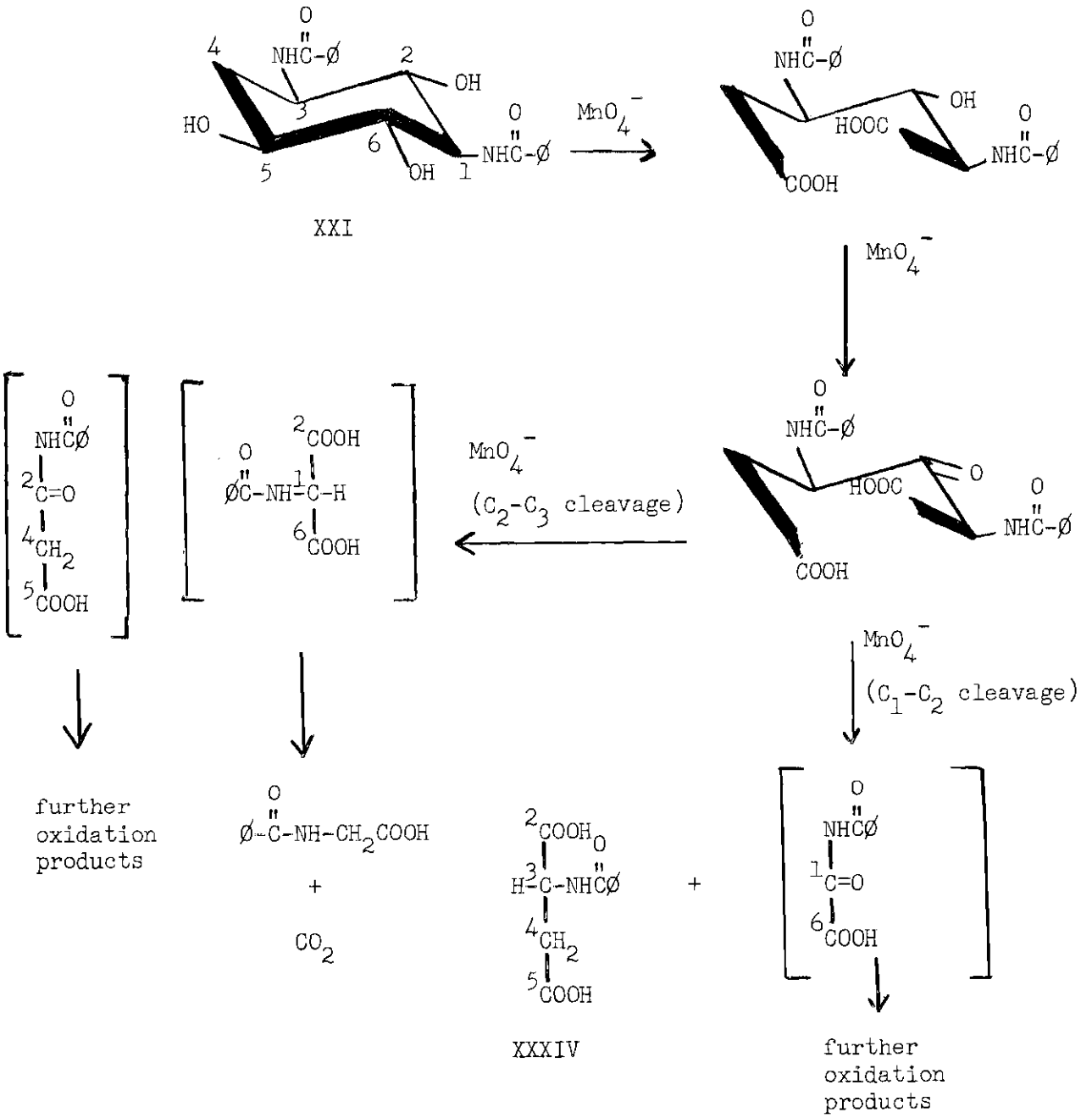
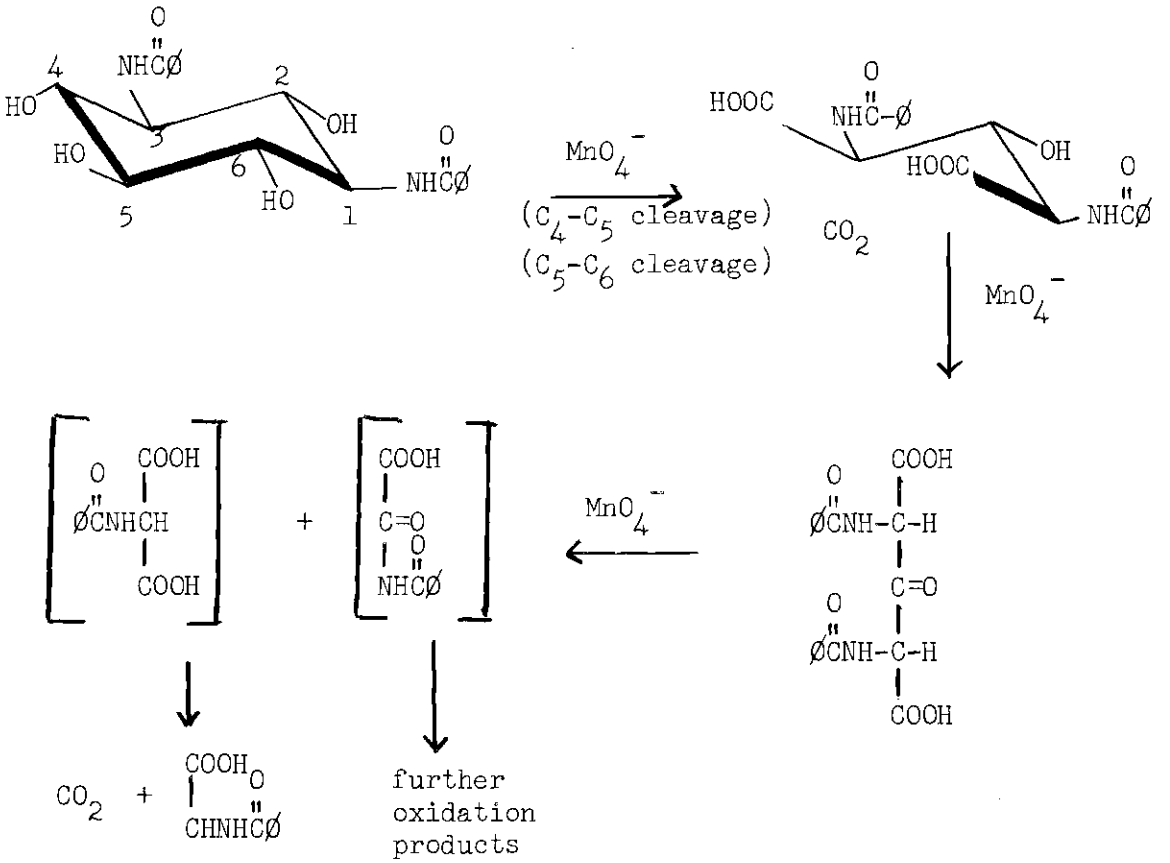


Fig. 3. Theoretical Degradation of N,N'-Dibenzoyl-4-deoxystreptamine to N-benzoyl-D- or L-Aspartic Acid.



XXXV

Fig. 4. Theoretical Degradation of N,N'-Dibenzoylstreptamine to N-Benzoylglycine.

permanganate, and water was allowed to stand at room temperature for 51 hr. Work-up gave a small amount of a light brown gum, which had an infrared spectrum very similar to that of benzamide. The fraction which would contain the acidic products of the reaction yielded a pale yellow gum. The gum was boiled under reflux with hydrochloric acid in order to hydrolyze any N-benzoylglycine to glycine. Evaporation of the hydrolysis

solution gave a gum which, upon paper chromatography, was found to contain no glycine. The other oxidation was run identically as described above except that a large excess of magnesium sulfate was added to the reaction mixture in order to insure neutrality during the reaction. Paper chromatograms of the gum obtained showed no trace of glycine.

Another oxidation was tried starting with 0.223 g. (0.000578 mole) of N,N'-dibenzoylstreptamine, 0.606 g. (0.00384 mole) of potassium permanganate, and 50 ml. of water. The solution was boiled under reflux for three hours. The manganese dioxide was filtered, and the filtrate was extracted with ether. The ether extracts yielded 0.144 g. of crude gummy solid. The material was recrystallized from 2 ml. of water; this gave 0.021 g. (0.00017 mole) of pure benzamide. Evaporation of the filtrate gave 0.070 g. of white crystals (probably benzamide) and waxy solid. The aqueous solution yielded 0.118 g. (0.00096 mole) of crude benzoic acid and about 0.04 g. of a green, waxy chloroform-insoluble solid, which was not further characterized. This means that 0.00113 moles of "benzoyl groups" (benzamide plus benzoic acid) were obtained from 0.00116 moles of benzoyl groups (two benzoyl groups per molecule of starting material). Even assuming a small amount of impurities in the benzoic acid isolated, few benzoyl groups would remain to be involved in hippuric acid. It thus appeared that the oxidation conditions were either degrading the molecule in some other way than that anticipated or were degrading any hippuric acid formed.

To test the latter hypothesis a solution of 0.110 g. (0.000615 mole) of authentic hippuric acid, 2.0 g. (0.013 mole) of potassium permanganate, 7.6 g. (0.063 mole) of magnesium sulfate, and 150 ml. of water

was boiled under reflux for 4 hr. The manganese dioxide(!) was removed by filtration and the filtrate was extracted with ether. The ether extracts yielded 0.050 g. (0.00041 mole), 67%, of benzamide (identified by m.p. and infrared spectrum). The aqueous solution was acidified with hydrochloric acid and extracted with ether. This gave 0.055 g. of brown, viscous liquid which was not further characterized.

Thus, it appeared unlikely that hippuric acid could be obtained from N,N'-dibenzoylstreptamine by means of permanganate oxidation. It was considered highly probable that similar attempts on N,N'-dibenzoyl-4-deoxystreptamine would likewise meet with failure. This line of attack was therefore abandoned.

The reaction sequence shown as Fig. 5 was conceived which would lead to D-serine (XXXVII) from one of the possible enantiomers (XXI) of N,N'-dibenzoyl-4-deoxystreptamine and to L-serine from the other. The serine could be isolated from the final mixture by use of ion-exchange resins.

The first step in the sequence, the conversion of the deoxy compound to 2,4-dibenzamido-3-hydroxyadipaldehyde (XXXVI), had been reported by Folkers and his associates (22). A very similar compound, 2,4-dibenzamido-3-hydroxyglutaraldehyde (XXXVIII), had been prepared from N,N'-dibenzoylstreptamine by Carter, Loo, and Rothrock (42) and oxidized to the corresponding di-acid (XXXIX) by means of bromine water. This offered a likely procedure for the second step in the proposed sequence.

Several drawbacks were anticipated for this sequence. In the first place, yields would have to be rather high since five steps are involved past the deoxy compound. This compound was on hand in rather small

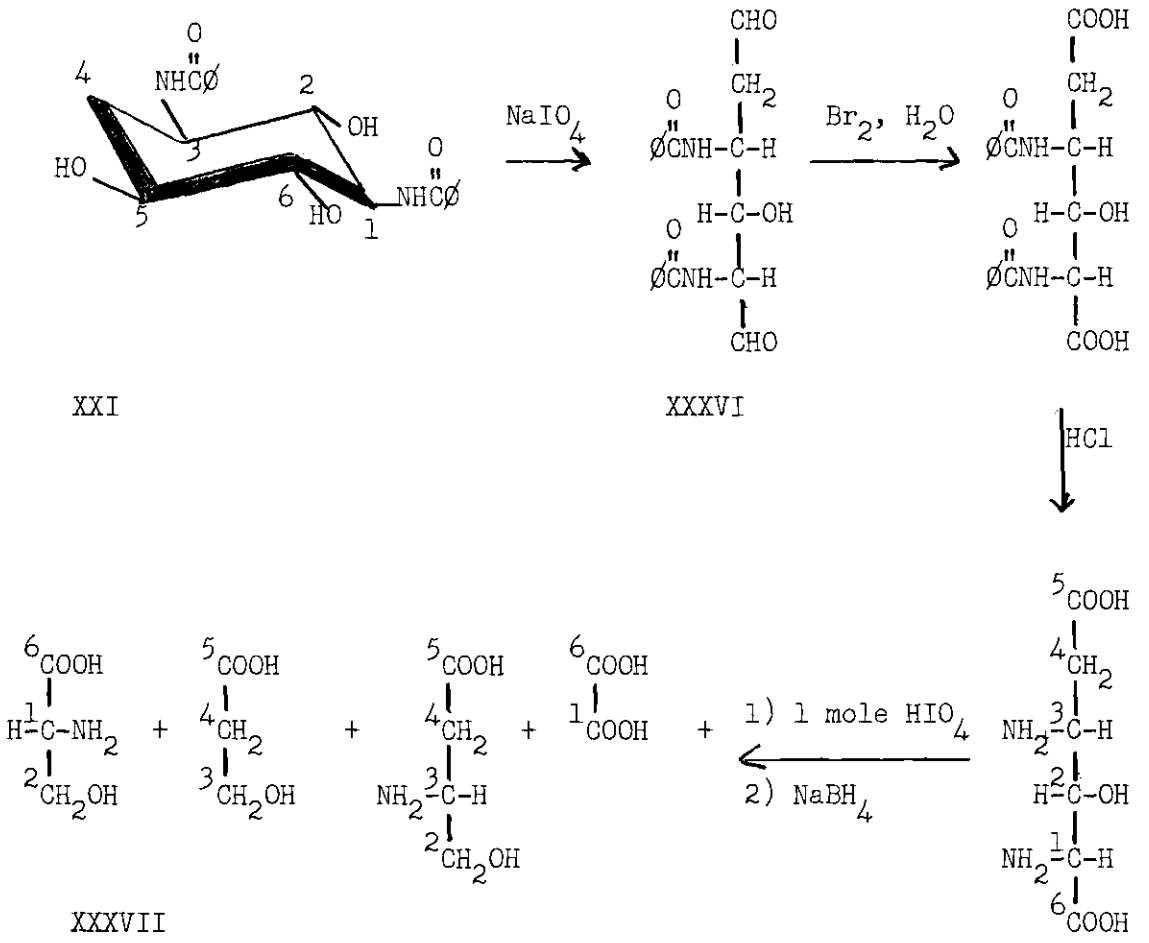
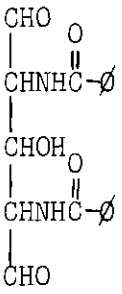


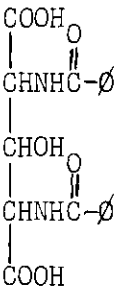
Fig. 5. Theoretical Degradation of N,N'-Dibenzoyl-4-deoxystreptamine to D- or L-Serine.

quantity and was very laborious and time-consuming to prepare. Secondly, the diaminohydroxydiacid could possibly cyclize in several ways which would cause the subsequent periodate oxidation to proceed in an undesirable manner.

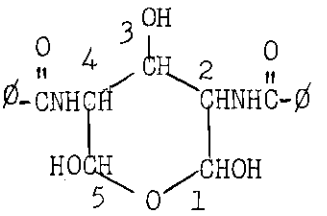
Before proceeding upon this sequence with N,N'-dibenzoyl-4-deoxystreptamine, the first two steps were carried out on the model compound,



XXXVIII



XXXIX

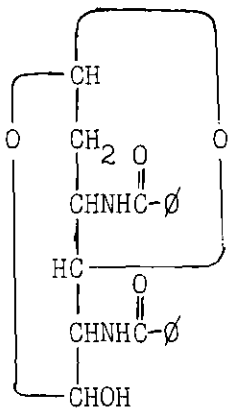


XL

N,N'-dibenzoylstreptamine, following Carter's procedures. No difficulty was encountered in obtaining 2,4-dibenzamido-3-hydroxyglutaraldehyde. Carter reported that the usual material, obtained in about 90% yield, melted at 130-131° but that occasional samples melted at 143-145° or 163-165°. All three materials gave the same hydrazone, which indicates that they are probably stereoisomers about C_1 and C_5 of the cyclic hemiacetal structure XL. Carter assigned structure XL to the product obtained because of its relative stability to catalytic oxidation, its analytical data indicative of dibenzamidohydroxyglutaraldehyde plus one mole of water, and because of its formation of a triacetyl derivative. We obtained a 70% yield of white crystals, m.p. 132-133°, and a 12% yield of white crystals, m.p. 164-165°. Infrared spectra of nujol mulls of the two compounds were very similar. However, the lower melting material showed a weak-to-medium aldehyde peak at 5.75 μ, whereas the higher melting compound showed no trace of a free aldehyde group. It is possible that some free aldehyde was present along with the cyclic structure, especially since the material was not further purified after isolation. The glutaraldehyde derivative was oxidized with bromine water to the corresponding di-acid in 32% yield. White crystals were obtained which melted at 192-193°. The reported

melting point and yield was 199-200° and 62%. An infrared spectrum of a nujol mull of the compound showed a broad carbonyl peak at 5.80 μ. An attempt to prepare the di-acid by use of Tollen's reagent was unsuccessful.

Preparation of 2,4-dibenzamido-3-hydroxyadipaldehyde was accomplished using the procedure of Folkers and his associates. The reaction was run in aqueous solution at room temperature using one mole of sodium metaperiodate per mole of N,N'-dibenzoyl-4-deoxystreptamine. White crystals of 2,4-dibenzamido-3-hydroxyadipaldehyde which melted at 142-144° were obtained in about 95% yield. The reported melting point and yield after one recrystallization were 148-149° and 33%. An infrared spectrum of the compound was taken (potassium bromide pellet). Peaks were observed at 2.95, 6.07, 6.21 (shoulder), 6.35, 6.56 μ, among others, in good agreement with the peaks reported for the compound. No peak was observed for an aldehyde carbonyl. Folkers has concluded on the basis of the infrared data and the stability of the compound that it likely has the cyclized structure XXIV.



XXIV

Three unsuccessful attempts were made to oxidize the adipaldehyde

using bromine water; no trace of the corresponding di-acid was found. Catalytic oxidation using oxygen and 5% platinum-on-carbon catalyst was also unsuccessful. Sodium hypoiodite oxidation also failed. The failure of the adipaldehyde to undergo oxidation is likely tied up in some manner with its cyclic structure. It was felt that further efforts to oxidize the compound would lead at best to a low yield of the di-acid. A low yield at that point of the degradation would be of little use. Consequently, this line of approach was abandoned in favor of a new one.

From 2,4-dibenzamido-3-hydroxyadipaldehyde Folkers and associates had prepared the tetraethyl mercaptal derivative (XLI) (22). Reduction of aldehydes to hydrocarbons through the action of Raney nickel on the mercaptal derivatives is not uncommon. It was theorized that the sequence shown as Fig. 6 would lead to D-2-aminopropan-1-ol from one enantiomer of N,N'-dibenzoyl-4-deoxystreptamine and to L-2-aminopropan-1-ol (XLII) from the other possible enantiomer. Authentic D- and L-2-aminopropan-1-ol could be obtained by lithium aluminum hydride reduction of D- and L-alanine or their esters. The aminopropanol obtained in the degradation could then be compared with the authentic stereoisomers to determine its stereochemistry and thus the absolute configuration about C_1 of N,N'-dibenzoyl-4-deoxystreptamine.

The tetraethyl mercaptal was prepared by suspending 2,4-dibenzamido-3-hydroxyadipaldehyde in ethanethiol saturated with hydrogen chloride gas at room temperature. Chromatography of the crude product over a column packed with acid-washed alumina and one recrystallization gave a 70% yield of crystalline 2,4-dibenzamido-3-hydroxyadipaldehyde, which melted at 144-145°. The reported melting point and yield were 146-146.5° and 48%.

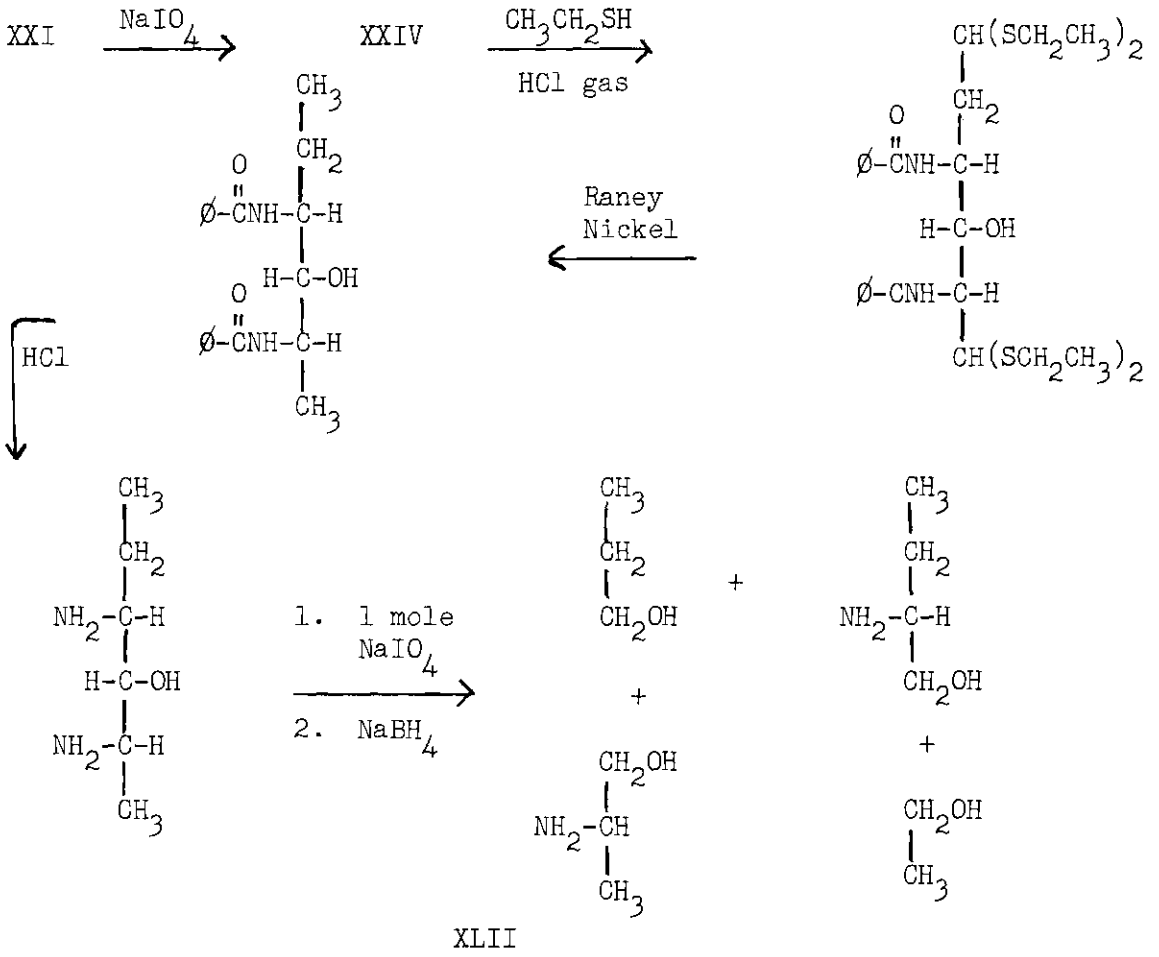


Fig. 6. Theoretical Degradation of N,N'-Dibenzoyl-4-deoxystreptamine to D- or L-2-Aminopropan-1-ol.

The infrared spectrum of a 1% solution of the compound in chloroform showed absorptions at 2.85, 2.95, 3.35, 6.04, 6.60, 6.72, and 6.90 μ, among others. These peaks were compatible with structure XLI for the compound. The n.m.r. spectrum of a 10% solution of the compound in deuterochloroform was taken. It showed complicated multiplets in the regions 1.8-2.8 τ (ArH; 10.0 H), 4.8-6.3 τ (C_1-H, C_2-H, C_3-H, C_4-H, and C_6-H; 4.7 H), 7.0-8.0 τ ($-CH_2-$ groups; 9.4 H), and 8.4-9.1 τ ($-CH_3$ groups; 12.0 H). Thus, the n.m.r. data also corroborate the structure shown for the tetraethyl mercaptal.

Attempted reduction of the tetraethyl mercaptal in 95% ethanol

under 1 atm. of hydrogen for 40 hr. using Raney nickel as catalyst led to the isolation of only starting material from the reaction mixture. The same result was obtained using dioxane for the solvent and 20 hr. reaction time. A mixture of the tetraethyl mercaptal, 70% ethanol, and Raney nickel boiled under reflux for two hours yielded only starting material. A mixture of the tetraethyl mercaptal, dioxane, and Raney nickel was allowed to react for 24 hr. under 40 p.s.i. (gage pressure) of hydrogen in a Parr pressure reaction apparatus. A small amount of insoluble white crystals was obtained, which did not melt up to 330°. Starting material was the only other substance found upon work-up.

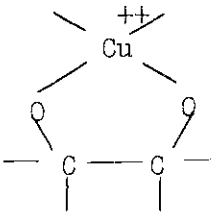
A mixture of 0.500 g. of tetraethyl mercaptal, 70% ethanol, and Raney nickel was boiled under reflux for 48 hr. with mechanical stirring. Work-up gave 0.183 g. of white crystals that did not melt up to 330° and 0.350 g. of starting material. The high-melting material was thought to be the desired 2,4-dibenzamido-3-hydroxyhexane. The analytical sample was recrystallized from acetic acid. The analysis checked very well for calcium acetate. A flame test and oxalate precipitate confirmed the presence of calcium. The original (unrecrystallized) material was examined to determine the anion present. The compound was very soluble in water and the solution was neutral; this ruled out the possibility of it being calcium oxide. A solution of the compound gave no precipitate with silver nitrate; this ruled out calcium halide. No brown ring was formed when ferrous sulfate and sulfuric acid were added to a solution of the compound; this ruled out calcium nitrate. A solution of the compound gave no precipitate with barium chloride; this (as well as the solubility of the compound in water) ruled out calcium sulfate, calcium carbonate, and calcium

phosphate. When an aqueous solution of the compound was made basic with ammonium hydroxide, no aluminum hydroxide precipitated; this ruled out calcium aluminate. It is thus difficult to imagine what this compound could be. That it is inorganic was shown by an ignition test. That it arose from the Raney nickel was confirmed by isolation of the usual amount of it from a blank reaction run identically as described above except that no tetraethyl mercaptal was used. Since the reduction attempts were fruitless, this method of attack was abandoned.

Reeves has developed a powerful method for the determination of the absolute configuration of many glycols (43). The method involves the measurement of the shift in optical rotation when an optically active glycol is complexed with "cuprammonium" -- a solution of ammonium hydroxide and copper(II) ion.

The reaction between cuprammonium and a glycol is a reversible, bimolecular association. It appears to occur instantaneously, which suggests that, initially at least, the reaction is ionic. The reaction is evidenced by spectrophotometric and conductimetric phenomena; and, with optically active glycols, is often accompanied by dramatic changes in optical rotation. The region of most pronounced change in absorption spectra lies in the near ultraviolet, between 300 and 400 mμ. The exact structure of the complex is unknown, but the complex is known to involve one molecule of glycol per molecule of cuprammonium. A five-membered ring is formed when the hydroxyl groups are on adjacent carbons (XLIII).

The composition of cuprammonium varies widely with ammonia concentration, and probably with hydroxyl ion concentration as well. Therefore, it is desirable to work with standard cuprammonium solutions. two



XLIII

extensively used standard solutions are designated Cupra A and Cupra B. The latter appears to be the more commonly used.

Adjacent groups attached to carbon atoms which form a part of a five-membered or a six-membered ring must be located, approximately, in one of the positions shown in Fig. 7. In these diagrams the lower of the two linked carbon atoms is presumed to be nearer the observer. The projection of the angle made by the two valence bonds onto a plane perpendicular to the carbon-carbon bond is called the projected angle between the groups under consideration. By convention this angle is negative if measured in a clockwise direction from the nearer group and positive if measured counterclockwise. Ring shapes will require the stable position of adjacent groups to be recognizably near one of the angles 0°, $\pm 60^{\circ}$, $\pm 120^{\circ}$, or 180°.

When cuprammonium combines with adjacent hydroxyl groups a new five-membered ring is formed. If the hydroxyls are located in the true cis position (0° projected angle) the copper-containing ring will be symmetrical, considering only the atoms of the new ring. Hydroxyls not in the true cis position yield asymmetric rings upon complexing with cuprammonium,

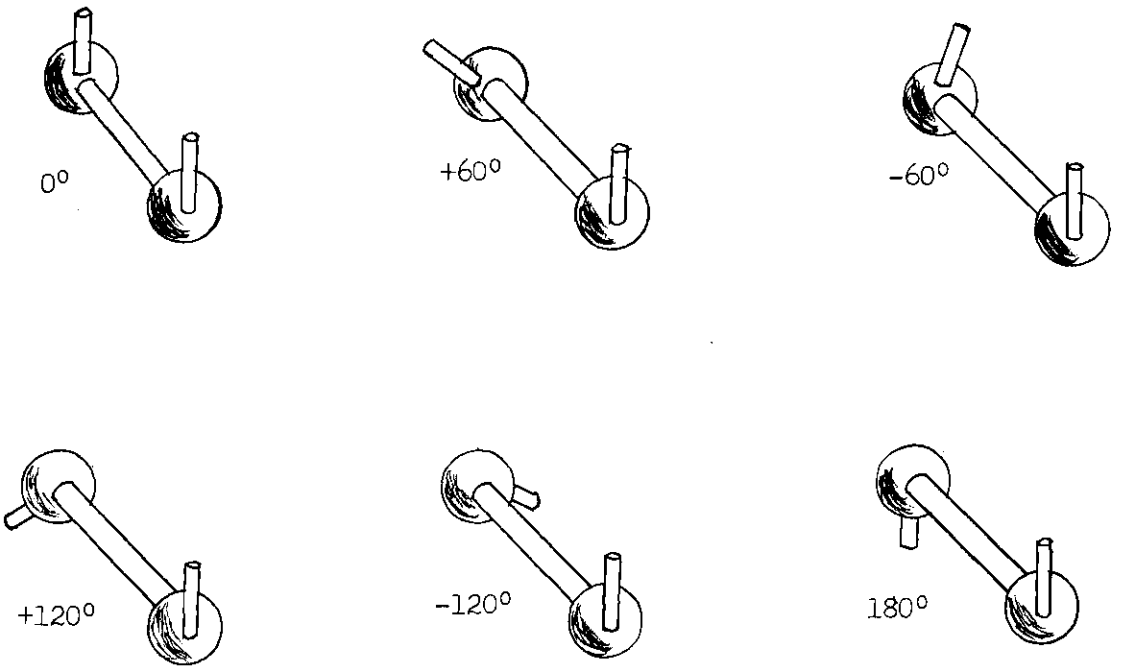


Fig. 7. The Various Angles between Groups Located on Adjacent Carbon Atoms.

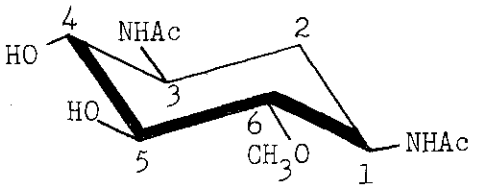
and the new rings exert a large rotational moment the sign of which is determined by the nature of the asymmetry. That is, if the projected angle between the hydroxyl groups is positive, the rotational shift will be positive; if negative, the rotational shift will be negative. This has been shown to be true in many examples using compounds of known configuration. The magnitude of the shift is, at least roughly, proportional to the extent of asymmetry. It has been well established that the complexing reaction will occur only at projected angles of 0° and 60° and not at 120° or 180°, thus limiting the method to glycols meeting this

projected angle requirement.

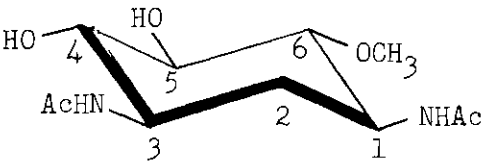
Complexing has also been observed with hydroxyl groups located in the 1,3 position, but only in the rare instances where the two oxygen atoms have been located by the shape of the molecule at a distance of not greater than about 3.4 Å.

Cuprammonium has been used extensively in the study of the structure of polysaccharides such as cellulose, starch, dextrins, mannans, and xylans (43). It has also been used in investigations of pyranoside rings (43). Recently, a communication was published which reported the determination of the absolute configuration of the 2-deoxystreptamine fragment of the neomycins, paromomycins, and kanamycins by use of cuprammonium (44). Rinehart and Hichens (44) prepared N,N'-diacetyl-6-O-methyl-2-deoxystreptamine from neomycin B. This compound was known to have either structure XLIV or structure XLV, which differ only in absolute stereochemistry (a problem remarkably similar to ours). In structure XLIV the hydroxyl groups on C_4 and C_5 form a projected angle of $+60^\circ$; in structure XLV the projected angle is -60°. The rotational shift of the cuprammonium complex of the compound would thus be positive if structure XLIV were correct and negative if structure XLV were correct. The observed shift was positive, thus establishing the absolute configuration shown in structure XLIV to be the correct one. 2-Deoxystreptamine would, of course, have the same absolute configuration as its derivative.

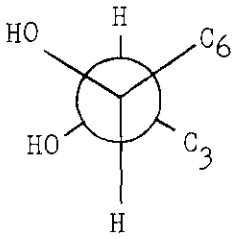
The cuprammonium method could certainly be applied to the problem at hand if a derivative of 4-deoxystreptamine with free hydroxyl groups at C_5 and C_6 could be found which would be sufficiently soluble in water and in cuprammonium to allow optical rotation measurements.

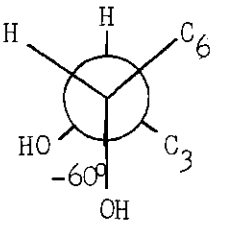


XLIV



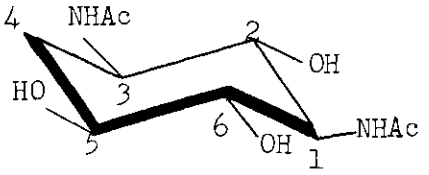
XLV



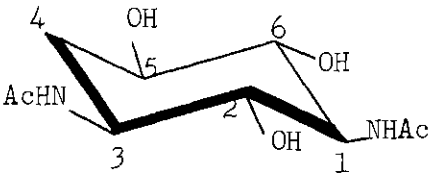


N,N'-Dibenzoyl-4-deoxystreptamine would not meet the solubility requirement, but if the corresponding diacetyl compound, XLVI or XLVII, could be obtained, it should be perfect for the determination of rotational shift. A rotational shift in cuprammonium in the negative direction would establish XLVI as the correct structure for N,N'-diacetyl-4-deoxystreptamine, and hence, structure Ib as the correct one for streptomycin. A positive shift would establish structures XLVII and Ia for the diacetyl compound and for streptomycin, respectively.

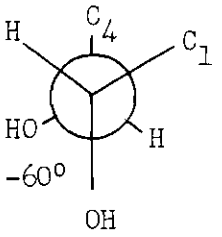
Although N,N'-diacetyl-4-deoxystreptamine had not been prepared previously, N,N'-diacetylstreptamine was a known compound, having been

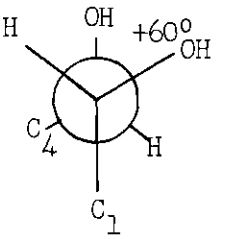


XLVI



XLVII





prepared from streptamine (11). Thus a good model procedure for the preparation of the former compound was at hand. Before proceeding with the preparation of the diacetyl-deoxy compound, the preparation of N,N'-diacetylstreptamine was attempted.

A mixture of streptamine sulfate, acetic anhydride, and sodium acetate was boiled under reflux for one hour. The acetic acid and excess acetic anhydride were removed by vacuum distillation and the residue was leached with hot chloroform. The filtrate was evaporated to dryness and the white crystals obtained were recrystallized. This gave a 35% yield of white crystals which partially melted at 250° and sublimed at 330-350°.

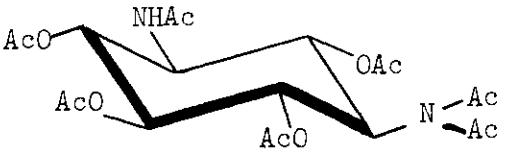
The reported yield was 32% and the reported melting behavior was partial melting at 240-247° with transition to longer needles and m.p. 342-345° in a sealed capillary.

The residue from the leaching of the reaction mixture with hot chloroform was extracted with chloroform for eight days in a Sohxlet apparatus. The extracts gave a total yield of 59% of white crystals which partially melted at about 250° with transition to longer needles and sublimed at 330-345°. The reported yield of this relatively chloroform-insoluble material was 70% and the reported melting point behavior was partial melting with transition to longer needles at 240-247° and m.p. 342-345° in a sealed capillary.

The difference in chloroform solubility is so great that the two compounds isolated must differ in some manner. The original workers did not comment on this difference.

Analytical data that we obtained on the two compounds were found to differ. The chloroform-soluble compound analyzed for heptaacetylstreptamine and the chloroform-insoluble compound analyzed for hexaacetylstreptamine. Infrared spectra of the two compounds as potassium bromide pellets are shown as Figures 8 and 9. Both spectra show peaks at 6.01 and 5.72 μ, assigned to the amide and ester carbonyl groups, respectively. The chloroform-soluble compound also shows a peak at 5.85 μ, while only a trace of that peak is present in the spectrum of the chloroform-insoluble compound. This peak was assigned to the imide carbonyl of heptaacetylstreptamine (XLVII). The n.m.r. spectrum of the chloroform-soluble heptaacetylstreptamine was taken in deuterochloroform. The protons of the acetyl groups (five peaks) absorbed at 7.7-8.2 τ and the ring protons absorbed as a

complicated series of peaks between 3.4 and t.2 τ.



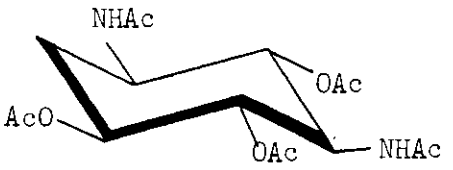
XLVIII

Thus, it is highly probable that the chloroform-soluble material isolated in this reaction was heptaacetylstreptamine and that the chloroform-insoluble compound was hexaacetylstreptamine.

A solution of hexaacetylstreptamine in absolute methanol was saturated with gaseous ammonia and left at room temperature for three hours. Work-up of the mixture gave a 77% yield of white crystals, m.p. 275-280°. The reported yield and melting point for N,N'-diacetylstreptamine were 66% and 283-284°.

Heptaacetylstreptamine, when treated similarly, gave a 68% yield of white crystals, m.p. 275-280°. The infrared spectra of both samples of N,N'-diacetylstreptamine were identical and showed absorption at 2.80, 3.00, 5.85, 6.18, and 6.40 μ, among others. The next step was the preparation of N,N'-diacetyl-4-deoxystreptamine. Pure N,N'-dibenzoyl-4-deoxystreptamine was hydrolyzed with 6N hydrochloric acid. Benzoic acid, 91%, was removed by chloroform extraction and the acidic solution was evaporated to dryness. The last traces of moisture were removed in vacuo. The 4-deoxystreptamine dihydrochloride was not characterized but was acetylated in the same manner used for the acetylation of streptamine sulfate. A 41%

yield of white crystals was obtained. A twice-recrystallized sample melted at 319-320°. Analytical data agreed with that calculated for pentaacetyl-4-deoxystreptamine (XLIX). No trace of a hexaacetyl derivative was found. If any were present, it should have been soluble in chloroform. The infrared spectrum of the compound was taken as a potassium bromide pellet and is shown as Figure 10. The spectrum was almost identical to that of hexaacetylstreptamine, as would be expected. Peaks at 5.74 and 6.03 μ were assigned to the ester and amide carbonyl groups, respectively.

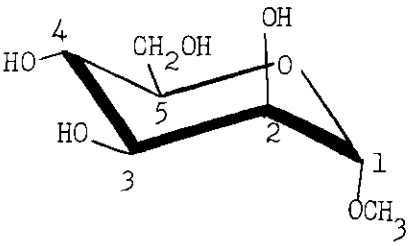


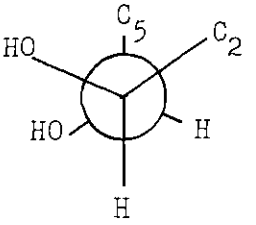
XLIX

A solution of pentaacetyl-4-deoxystreptamine in dry methanol at 0° was saturated with ammonia gas. The solution was left at room temperature for three hours. Work-up of the reaction mixture gave a 32% yield of white crystals, m.p. 297-298°. The optical rotation at the sodium D line was +5° in water at 29°. Analytical data agreed with that calculated for N,N'-diacetyl-4-deoxystreptamine (XLVI or XLVII). The infrared spectrum was taken as a potassium bromide pellet and is shown as Figure 11. The spectrum was almost identical to that of N,N'-diacetylstreptamine, as would be expected.

It was decided to use the standard cuprammonium solution designated "Cupra B" for the complexing solution. Cupra B contains 15.0 ± 0.1 g. (0.235 mole) of copper ion, 240 ± 5 g. (14.1 mole) of ammonia, and 1 g. of glycerol per liter (43).

Before applying the method to N,N'-diacetyl-4-deoxystreptamine, it was thought best to test our technique and apparatus by determining the optical rotation in water and in Cupra B of methyl α-D-mannopyranoside (L). The values for the optical rotations and also the rotational shift for this compound have been reported (49). The rotational shift caused by cuprammonium complexing should be positive for this compound since the C_3 and C_4 hydroxyls involved in the complex formation form a projected angle of +60°.





L

The specific rotation at 436 mμ for a solution of pure methyl α-D-mannopyranoside in water (c 0.57) was found to be +153 ± 5°. The reported value was 147 ± 5°. The specific rotation at 436 mμ for a solution of the compound in Cupra B (c 0.587) was found to be +1005 ± 120°. The reported value was +1050° (c 0.55). The rotational shift is calculated by use of the formula, $\Delta[M]_{\text{Cupra B}} = ([\alpha]_{436,\ \text{Cupra B}} - [\alpha]_{436,\ \text{water}})$ Mol. Wt./100. The rotational shift calculated from the above data would be (+857 ± 125°)

194.2/100 or +1665 ± 243°. The reported value is +1752°. Thus, it appeared that the technique and apparatus used were certainly adequate.

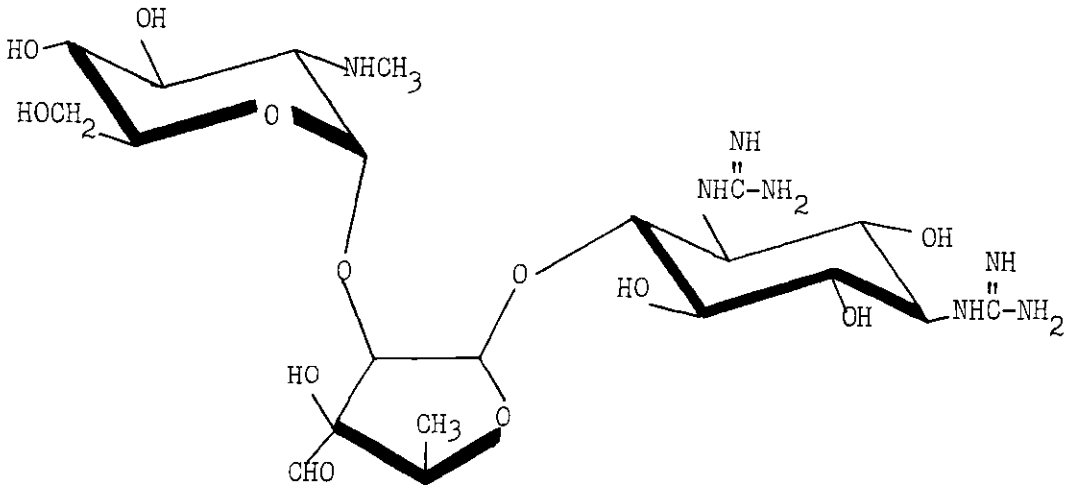
The procedure was then applied to N,N'-diacetyl-4-deoxystreptamine. The specific rotation at 436 mμ of a water solution (c 0.971) of the compound was found to be +5 ± 9°. A total of six determinations of the specific rotation at 436 mμ was made on two Cupra B solutions of the compound of concentration 0.880 and 0.700, respectively. The values found were: -886 ± 90°, -932 ± 100°, -1000 ± 55°, -1000 ± 214°, -928 ± 86°, and -1070 ± 70°. The average value was -970 ± 60°. There was no doubt that the rotational shift in Cupra B was negative. The value calculated was $\Delta[M]_{\text{Cupra B}}$ = (-975 ± 69°) 246.3/100 or -2400 ± 170°. The strong negative increment is similar to that obtained for the 2,3-glycol complex of D-glucosides ($\Delta[M]_{\text{Cupra B}} \approx$ -2075°) but opposed to that obtained for the 3,4-glycol complex of D-glucosides ($\Delta[M]_{\text{Cupra B}} \approx$ +2150°) (43). Interferences from a potential trans 1,3-glycol complex are not observed (43), and interference from acetamido groups does not occur (44).

Since the rotational shift was negative, the projected angle formed by the hydroxyl groups on C_5 and C_6 of N,N'-diacetyl-4-deoxystreptamine must be -60°. The compound must have the absolute stereochemistry shown in structure XLVI and not that in structure XLVII. Therefore, streptomycin must have structure Ib (and not Ia) in which the absolute configuration of the carbon atoms of the streptidine ring is 1(R), 2(R), 3(S), 4(R), 5(R), 6(S) (23). This assignment is in agreement with the suggestion of Tatsuoka (24).

This stereochemical result also establishes the complete structure determination of dihydrostreptomycin, hydroxystreptomycin, and

mannosidostreptomycin. The configurational determination of the asymmetry of the streptidine ring in streptomycin yields the same result (i.e., R at C_4) as that recently determined chemically (24) and by the cuprammonium method (44) for the 2-deoxystreptamine ring of the neomycins, paromomycins, and kanamycins. Thus these two vital components of a number of powerful antibiotics may have stereochemically similar biogenetic precursors.

A brief account of this work has been published (45).



Ib

Streptomycin

CHAPTER III

EXPERIMENTAL

Apparatus and Techniques

Unless otherwise indicated, melting points were determined using a Kŏfler hot stage and are corrected. Microanalyses were prepared by Galbraith Laboratories (Knoxville, Tennessee) and by Huffman Laboratories (Wheatridge, Colorado). Infrared spectra were determined using a Perkin Elmer Model 137 Infracord recording spectrophotometer.

The nuclear magnetic resonance spectra were determined using a Varian Model A-60 spectrometer. All values quoted are on the τ scale, relative to tetramethylsilane as an internal standard.

Optical rotations were determined using a Bellingham and Stanley Model No. 397619 polarimeter. A General Electric Sodium Lab-Arc lamp was the source of the sodium D line (589 mμ). A General Electric mercury lamp was used in conjunction with standard thickness Corning filters 3389 and 5113 to provide the mercury blue line (436 mμ).

Qualitative color tests used were the ninhydrin test (46) and Nessler's test (47).

The apparatus and techniques used in paper chromatography were the same as those described previously (48). The solvent systems that were used and their abbreviations are : τ-butyl alcohol-acetic acid-water, 2:1:1 (v/v), (BAW); n-butyl alcohol-6N hydrochloric acid solution, 7:3 (v/v), BH); ethanol-water, 7:1 (v/v), (EW).

Unless otherwise indicated, evaporations were carried out under

reduced pressure using a rotating evaporator.

Polybenzoyldihydrostreptomycin

To 75.0 g. (0.103 mole) of dihydrostreptomycin sulfate (Merck lot No. 01B4185, used as received) was added 1.7 l. of chloroform and 575 ml. of pyridine (Eastman 214, used as received); the mixture was stirred mechanically for 30 min while being cooled in an ice-water bath. With continued cooling and stirring 700 ml. (6.25 mole) of benzoyl chloride (Eastman 293, used as received) was added slowly over a two-hour period. The mixture was allowed to warm to room temperature.

The clear, dark red solution was washed successively with aqueous sodium bicarbonate solution, 5% sulfuric acid, and water. The organic layer was dried with anhydrous magnesium sulfate and concentrated to ca. 1 l. The solution was poured with stirring into ca. 3 l. of petroleum ether (Matheson, Coleman and Bell PX425, used as received). A gummy precipitate formed. The precipitate was dissolved in 800 ml. of chloroform and the solution that resulted was divided into 100 ml. portions. Each portion was poured slowly with stirring into separate 2 l. portions of petroleum ether; a white precipitate formed immediately. The mixture was filtered and the residue was dried in air for 24 hr. The yield was 195 g., 106% (based on dodecabenzoyldihydrostreptomycin; yields varied from 92 to 106%), of a buff-colored, amorphous solid, m.p. 137-141°, $[\alpha]_D$ +83 ± 2° (c 2.0, chloroform); [lit. (21) $[\alpha]_D$ +51° (c 1.78, chloroform].

For analysis a small amount of the compound was dissolved in chloroform and precipitated from petroleum ether to yield a white, amorphous compound, m.p. 141-145°, $[\alpha]_D^{25}$ +85 ± 2° (c 2.0, chloroform).

Anal. $C_{21}H_{29}N_7O_{12}(C_7H_5O)_{12}$ (1833) Calcd.: C, 68.81; H, 4.91; N, 5.35

$C_{21}H_{30}N_7O_{12}(C_7H_5O)_{11}$ (1729) Calcd.: C, 68.11; H, 4.95; N, 5.68

$C_{21}H_{31}N_7O_{12}(C_7H_5O)_{10}$ (1625) Calcd.: C, 67.26; H, 5.02; N, 6.04

Found: C, 67.62; H, 5.14; N, 5.24

Heptabenzolystreptidine

To 70.0 g. (0.0405 mole, assuming undecabenzoyldihydrostreptomycin) of polybenzoyldihydrostreptomycin dissolved in 1.33 l. of chloroform was added 32 ml. of 32% hydrogen bromide in acetic acid (Eastman 1161, used as received). The solution was left at room temperature for ca. 22 hr.

The solution was washed with aqueous sodium bicarbonate and then with water. The organic layer was dried with anhydrous magnesium sulfate and evaporated to ca. 100 ml. To the residue was added 300 ml. of dry benzene and the mixture was boiled under reflux for 2 hr. About 1.5 l. of methanol was added to the hot solution which was then refrigerated overnight.

The mixture was filtered and the residue was dried in air. The white, amorphous solid weighed 27.8 g., 70% (yields varied from 54 to 70%); m.p. 250-252°, $[\alpha]_D^{25}$ +53° ± 2° (c 1.0, chloroform); [lit. (21) m.p. 256-258°, $[\alpha]_D^{25}$ +54° (c 1, chloroform)].

For analysis a small amount of the solid was recrystallized from 1-butanol and then from dioxane-petroleum ether. The recrystallized solid had m.p. 251-252°.

Anal. $C_8H_{11}N_6O_4(C_7H_5O)_7$ (991.0) Calcd.: C, 69.08; H, 4.68; N, 8.46
Found : C, 68.75; H, 4.87; N, 8.25

Heptabenzoyl-4-O-mesylstreptidine

A solution of 49.0 g. (0.0495 mole) of heptabenzoylstreptidine and 640 ml. of pyridine was cooled to ca. 5°. To the cold solution was added 49 ml. (0.65 mole) of methanesulfonyl chloride (Eastman 5388, redistilled, b.p. 160°), and the solution was refrigerated at ca. 5° for 18 hr. The dark solution was placed in an ice-water bath and 50 ml. of water was added dropwise with stirring. The solution was poured into 1.2 l. of water; this resulted in the precipitation of a gummy solid. The mixture was extracted four times with a total of 1.5 l. of chloroform, and the combined chloroform extracts were washed successively with 10% hydrochloric acid, aqueous sodium bicarbonate, and water. The organic layer was dried with anhydrous magnesium sulfate and concentrated to ca. 200 ml. The concentrate was heated to boiling and 300 ml. of methanol was added slowly with continued heating; the solution was refrigerated overnight.

The mixture was filtered and the residue was dried in air. The yield was 47.% g., 89% (yields varied from 78 to 89%), of white crystals, m.p. 241-242°, $[\alpha]_D^{25}$ +15 ± 2° (c 0.8, chloroform). The compound was recrystallized from chloroform-methanol; m.p. 241-242°, $[\alpha]_D^{25}$ +16 ± 2° (c 0.8, chloroform); [lit. (22) m.p. 241.5-242°, $[\alpha]_D^{25}$+18° (c 0.8, $HCCl_3$)].

Heptabenzoyl-4-iodostreptidine

To 9.0 g. (0.0084 mole) of heptabenzoyl-4-O-mesylstreptidine contained in a 400 ml. hydrogenation bottle was added 22.5 g. (0.15 mole)

of sodium iodide (Baker 3748, dried at 110° for 4 hr.) and 160 ml. of acetone (Eastman 297, used as received). The bottle was clamped securely in a metal vise in order to hold the rubber stopper when heating caused a rise in pressure. The apparatus was placed in a steam bath at ca. 85° for 4 hr.

The reaction mixture was cooled to below room temperature before removal of the vise and stopper. To the reaction mixture was added 250 ml. of 50% chloroform-water. The layers were separated and the aqueous layer was extracted four times with a total of 100 ml. of chloroform. The combined chloroform extracts were washed successively with aqueous sodium bicarbonate, aqueous sodium thiosulfate until the iodine color disappeared, and water. The solution was dried with anhydrous magnesium sulfate and then evaporated to dryness. The crude white residue, m.p. 143-145°, $[\alpha]_D^{25}$ +21 ± 4° (c 1.0, chloroform), weighed 11 g., 118% (yields varied from 96 to 118% based on pure heptabenzoyl-4-iodostreptidine).

Crude heptabenzoyl-4-iodostreptidine, 59 g., was purified by chromatography through a 6.5 cm. diameter column containing 1 kg. of silicic acid in chloroform. Chloroform was used as the eluting agent. The residue from fractions 1-9 (8900 ml., ca. 1 g. of yellow gum) was discarded. The residue from fractions 10-13 (3250 ml., 29.3 g. of pale yellow solid) was dissolved in chloroform. The solution was added slowly to 900 ml. of methanol; 24.1 g. of white, amorphous solid precipitated; m.p. 146-147°, $[\alpha]_D^{25}$ +40 ± 2° (c 1.8, chloroform; [lit. (22) m.p. 153-154°, $[\alpha]_D^{25}$ +23° (c 0.08, chloroform)]. A second crop weighed 1.00 g., m.p. 145-146°. The residue from fractions 14-16 (700 ml., 7.83 g. of purple-tinged solid) was dissolved in 25 ml. of chloroform, and the solution was added

slowly to 300 ml. of methanol; 5.5 g. of white, amorphous solid precipitated, m.p. 146-147°. A second crop weighed 0.53 g., m.p. 143-145°. The residue from fractions 17-19 (1825 ml., 10.6 g. of brown solid) was set aside for rechromatography. The total yield of compound melting at ca. 145° was 31.1 g., 56%.

For analysis a small amount of compound was recrystallized from carbon tetrachloride. The sample had m.p. 154-155°. It showed $[\alpha]_D$ +15.8° (c 0.16, chloroform); [lit. (22) $[\alpha]_D^{25}$ +23° (c 0.08, chloroform)].

Anal. $C_8H_{10}N_6O_3I(C_7H_5O)_7$ (1101) Calcd.: C, 62.18; H, 4.12; N, 7.63; I, 11.53

Found : C, 62.03; H, 4.05; N, 7.37; I, 11.22

Heptabenzoyl-4-deoxystreptidine

To a solution of 4.0 g. (0.0036 mole) of heptabenzoyl-4-iodostreptidine and 150 ml. of 90% dioxane (Eastman 2144, used as received)-water was added ca. 30 ml. of moist Raney nickel No. 28 catalyst (Raney Catalyst Co., Chattanooga, Tenn., prepared by six washings with water and one with 90% dioxane-water). The reaction mixture was subjected to hydrogenolysis under 48 p.s.i. (gage pressure) of hydrogen for 8 hr. using a Parr Pressure Reaction Apparatus No. 3911.

The catalyst was removed by filtration and the filtrate was evaporated to dryness. The product (ca. 4 g. of pink-white solid) was dissolved in 70 ml. of hot chloroform and 100 ml. of methanol was added with heating and stirring; the mixture was refrigerated for several hours. The mixture was filtered and the residue was washed with methanol. A very small amount of the white solid was dried in air; m.p. 191-193°.

The remainder was dissolved in 40 ml. of hot ethyl acetate and 20 ml. of methanol was added with heating. The solution was refrigerated for several hours and then filtered. The white, amorphous solid, m.p. 198-199°, $[\alpha]_D^{25}$ +47 ± 3° (c 2.0, chloroform) [lit. (22) m.p. 198-199°], weighed 1.6 g.

The filtrate from the chloroform-methanol precipitation yielded a second crop of white solid, m.p. 165-175°. This crop was reprecipitated from 25 ml. of ethyl acetate and 40 ml. of methanol; this gave 0.46 g. of white, amorphous solid, m.p. 191-193°.

The filtrates from the two ethyl acetate-methanol reprecipitations were combined and evaporated to dryness. To the residue was added 12 ml. of hot ethyl acetate and 35 ml. of hot methanol. The solution was refrigerated and then filtered; the residue was washed with methanol and dried in air. The white, amorphous solid, m.p. 190-192°, weighed 0.63 g. The total yield was 2.7 g., 76% (yields varied from 45 to 76%).

For analysis a small amount of the compound was reprecipitated from chloroform-methanol. The sample melted at 198-199°.

Anal. $C_8H_{11}N_6O_3(C_7H_5O)_7$ (976) Calcd.: C, 70.21; H, 4.76; N, 8.62
Found : C, 70.07; H, 4.74; N, 8.34

Pentabenzoyl-4-deoxystreptamine

A mixture of 8.5 g. (0.0087 mole) of heptabenzoyl-4-deoxystreptidine, 4.0 l. of methanol (redistilled from magnesium turnings, b.p. 64°), and 4.42 g. (0.026 mole) of barium hydroxide (Baker 1006, dried at 120° for 12 hrs.) was stirred mechanically at ca. 25° for 22 hr. The solution was saturated with carbon dioxide and evaporated to dryness. The residue was leached with 550 ml. of hot 50% methanol-water; the mixture was filtered

and the filtrate evaporated to dryness.

The residue (4-deoxystreptidine carbonate) was boiled under reflux for 30 hr. with 550 ml. of saturated aqueous barium hydroxide. The solution was concentrated to ca. 275 ml. Hydrochloric acid was added until the solution was distinctly acidic, and the solution was evaporated to dryness. The residue was leached with 800 ml. of hot methanol; the mixture was filtered and the filtrate was evaporated to dryness. The residue was leached with 250 ml. of hot methanol; the mixture was filtered and the filtrate was evaporated to dryness.

To the residue (4-deoxystreptamine dihydrochloride) was added 282 ml. of pyridine, and the mixture was cooled to ca. 5° in an ice-water bath. With continued cooling and stirring 50 ml. (0.45 mole) of benzoyl chloride was added dropwise during 1 hr. The mixture was stirred at ca. 25° for 16 hr.

To the reaction mixture was added 300 ml. of chloroform; the solution was washed successively with aqueous sodium bicarbonate, 10% sulfuric acid, and water. The organic layer was dried with anhydrous magnesium sulfate and evaporated to ca. 60 ml. of dark sirup. The sirup was dissolved in 100 ml. of chloroform, and this solution was added slowly with stirring to ca. 4 l. of petroleum ether (b.p. 30-60°). The mixture was filtered; the residue was washed with petroleum ether and dried in air. This gave a yellow, amorphous solid, m.p. 250-280° dec. [lit. (22) m.p. 293-294°], which weighed 5.0 g., 77% (yields varied from 49 to 77%).

N,N'-Dibenzoyl-4-deoxystreptamine

A mixture of 6.6 g. (0.0089 mole) of crude pentabenzoyl-4-deoxystreptamine, 4.2 l. of methanol (distilled from magnesium turnings, b.p.

64°), and 10.6 g. (0.062 mole) of barium hydroxide (Baker 1006, dried at 120° for 12 hr.) was stirred at ca. 25° for 30 min. The solution was then refrigerated at ca. 5° for 16 hr., saturated with carbon dioxide, and evaporated to dryness.

The residue from five reactions carried out as described above was leached with 2 l. of hot 50% methanol-water; the mixture was filtered and the filtrate was evaporated to dryness. The residue was leached with 1 l. of hot methanol; the mixture was filtered and the filtrate was concentrated to ca. 100 ml. To the concentrate was added 50 ml. of methanol and 100 ml. of chloroform; the solution that resulted was chromatographed on a 5.0 cm. diameter column containing 900 g. of acid-washed alumina (Merck 71695, used as received). A solution of 33% methanol-chloroform was used to prepare the column and as the first eluting agent.

The residue from fractions 1 and 2 (325 ml., 0.4 g. of yellow gum) was discarded. The residue from fractions 3-8 (2150 ml., 14.5 g. of yellow-brown solid) was recrystallized from 500 ml. of hot methanol; this yielded 2.90 g. of white crystals, m.p. 277-279°. A second crop weighed 0.63 g., m.p. 279-281°, and a third, 0.58 g., m.p. 274-276°. The eluting agent was changed to 100% methanol. The residue from fractions 8-12 (2500 ml., 3.62 g. of yellow-white solid) was recrystallized from 500 ml. of methanol; this gave 1.16 g. of white crystals, m.p. 281-284°. A second crop weighed 0.37 g., m.p. 279-281°, and a third, 0.46 g., m.p. 279-281°. The total weight of recrystallized material from fractions 3-12 was 6.10 g., 37%. The residue from fractions 13-15 (1500 ml., 0.31 g. of yellow solid and orange gum) was set aside for rechromatography. The residue from fractions 16-18 (4000 ml., 0.55 g. of brown gum) was discarded.

For analysis a small amount of the compound was recrystallized twice from methanol. The sample melted at 284-286°; [lit. (22) m.p. 287-289°, $[\alpha]_D^{25}$ -4° (c 1.1, 50% acetic acid)].

Anal. $C_{20}H_{22}N_2O_5$ (370.4) Calcd.: C, 64.86; H, 5.98; N, 7.57
Found : C, 64.82; H, 5.81; N, 7.54

N,N'-Dibenzoylstreptamine

The procedure of Carter, Loo, and Rothrock (42) was used to prepare N,N'-dibenzoylstreptamine. From 100 g. (0.365 mole) of crude streptamine sulfate there was obtained 30 g. (0.078 mole), 21%, of white crystals, m.p. 280-285° dec. [lit. (42) m.p. 293-295° dec.].

Attempted Oxidation of N,N'-Dibenzoylstreptamine to Glycine Nitric Acid

A solution of 1.04 g. (0.00270 mole) of N,N'-dibenzoylstreptamine, 25 ml. of redistilled acetic acid (b.p. 118°), and 25 ml. of concentrated nitric acid was heated under reflux for 8 hr. using a steam bath; brown fumes appeared after 5 min. heating. The solution was evaporated to dryness; three portions of 25 ml. each of water were successively added and evaporated; 10 ml. of absolute ethanol was added and evaporated. This resulted in the isolation of 0.742 g. of pale yellow solid. The solid was recrystallized from water; this gave 0.400 g. (0.00328 mole), 61%, of white crystals, m.p. 123° (sealed capillary), which had an infrared spectrum identical to that of benzoic acid. The recrystallization filtrate was evaporated to dryness. This gave 0.180 g. of yellow gum that gave a positive ninhydrin test and a negative Nessler's test. A small amount of the gum was dissolved in water and chromatographed on paper

(BAW solvent system) along with streptamine and glycine. Streptamine gave an orange-yellow spot at R_F 0.34, glycine gave a purple spot at R_F 0.69, and the gum gave a faint, purple spot at R_F 0.65. Oxidations using reaction times of 46 hr. and 3.5 hr. gave similar results.

An oxidation of 0.998 g. (0.00259 mole) of N,N'-dibenzoylstreptamine carried out using a 6 hr. reaction time gave 0.523 g. (0.00428 mole), 83%, of benzoic acid. An aqueous solution of the chloroform-insoluble products was passed over a column of IR-45 ion exchange resin (hydroxyl phase). The eluate and washings were evaporated to dryness. A yellow gum was obtained that weighed 0.106 g. and gave a negative ninhydrin test. There was also obtained 0.028 g. of gum in a later fraction that gave a positive ninhydrin test. Elution of the column with 1N hydrochloric acid gave 0.120 g. of yellow gum that gave a positive ninhydrin test and was largely insoluble in water. A paper chromatogram of the two ninhydrin-positive products along with glycine gave in BAW: glycine, purple, R_F 0.53; first ninhydrin-positive material, red-orange, 0.39, purple, 0.44, orange, 0.49, purple, 0.76; second ninhydrin-positive material, orange, 0.29, pink, 0.47, purple, 0.55, gray-purple, 0.74.

Silver Permanganate

A solution of 0.695 g. (0.00180 mole) of N,N'-dibenzoylstreptamine, 2.84 g. (0.0125 mole) of silver permanganate, and 150 ml. of water was boiled under reflux for 8 hr. The mixture was filtered while hot to remove manganese dioxide and silver oxide. The filtrate was refrigerated for several hours and then filtered. A white solid, m.p. 283-285° dec., that weighed 0.067 g. (0.00017 mole) was obtained. Its appearance and melting point identified it as starting material. The filtrate was

adjusted to pH > 10 using aqueous sodium hydroxide and the solution was extracted five times with ether. The ether was evaporated; this gave 0.155 g. (0.00128 mole), 35%, of yellow solid, m.p. 124-128°, identified by its infrared spectrum and melting point as benzamide.

The aqueous solution was acidified to pH 1 with sulfuric acid and extracted five times with ether. The ether was evaporated; this gave ca. 0.5 g. of yellow liquid, which was very slightly soluble in 1 ml. of boiling water. An estimated 10 mg. of white solid, m.p. ca. 70°, precipitated upon refrigeration of the yellow liquid-water mixture.

Potassium Permanganate at 25°

To a solution of 0.190 g. (0.000492 mole) of N,N'-dibenzoylstreptamine in 500 ml. of water was added 0.405 g. (0.00256 mole) of potassium permanganate. The solution was left at 25° for 51 hr. Not quite all of the permanganate color was gone at the end of that period, but a large amount of manganese dioxide was present. The remaining permanganate was reduced with sodium bisulfite and the mixture was filtered. The filtrate was extracted five times with ether and the extracts were combined and evaporated to dryness. A light brown, gummy residue that weighed 0.020 g. was obtained. Its infrared spectrum was very similar to that of benzamide.

The aqueous solution was acidified to pH 1 with hydrochloric acid and extracted five times with ether. The ether extracts, when evaporated, gave 0.052 g. of pale yellow gum. The gum was boiled under reflux for 4 hr. with 25 ml. of 3N hydrochloric acid. The solution was evaporated to dryness and 2 ml. of water was added to the gummy residue. Paper chromatograms run on this solution beside a sample of glycine showed a faint, pink spot at R_F 0.05 and a brown spot at R_F 0.17 (glycine gave a purple

spot at R_F 0.22) in EW solvent system. In BAW a gray spot at R_F 0.16 and a red-orange spot at R_F 0.28 were found (glycine gave a purple spot at R_F 0.42).

Another reaction was performed identically as described above except 1.54 g. (0.0128 mole) of magnesium sulfate was added to the reaction mixture. From the first ether extracts 0.046 g. of yellow gum was obtained and from the second extracts, 0.047 g. of pale yellow gum. The gum was treated with hydrochloric acid. Paper chromatograms of the gum from the hydrochloric acid treatment showed exactly the same spots as described above.

Hot Potassium Permanganate Solution

A solution of 0.223 g. (0.000578 mole) of N,N'-dibenzoylstreptamine, 0.606 g. (0.00384 mole) of potassium permanganate, and 50 ml. of water was boiled under reflux for 3 hr. The manganese dioxide was filtered and the filtrate was extracted five times with ether. A total of 0.144 g. of yellow solid (plus a small amount of gum) was obtained by evaporating the ether. The material was recrystallized from water to give 0.021 g. (0.00017 mole) of pure benzamide, m.p. 125-126°. The filtrate was evaporated; this yielded 0.070 g. of waxy solid and crystals.

The aqueous solution was acidified to pH 2 with hydrochloric acid and extracted twice with petroleum ether. The petroleum ether was evaporated; this gave 0.050 g. (0.00041 mole) of white crystals, m.p. 123° (sealed tube), identified as benzoic acid. The aqueous solution was extracted five times with ether. Evaporation of the extracts gave 0.145 g. of gum. About 5 ml. of chloroform was added and part of the gum dissolved. The mixture was filtered and a residue of green, waxy solid was obtained

that weighed ca. 0.040 g. The filtrate was evaporated; this gave 0.068 g. (0.00056 mole) of benzoic acid.

Permanganate Oxidation of Hippuric Acid

A mixture of 0.110 g. (0.000615 mole) of hippuric acid, 2.0 g. (0.013 mole) of potassium permanganate, 7.6 g. (0.063 mole) of magnesium sulfate, and 150 ml. of water was boiled under reflux for 4 hr. The manganese dioxide was removed by filtration, and the filtrate was extracted five times with ether. The ether extracts, when evaporated, gave 0.050 g. (0.00041 mole), 67%, of benzamide. The aqueous solution was acidified with hydrochloric acid and extracted with ether. A brown, viscous liquid that weighed 0.055 g. was obtained. It was not characterized.

Attempted Oxidation of N,N'-Dibenzoyl-4-deoxystreptamine to Aspartic Acid

A solution of 0.500 g. (0.00135 mole) of N,N'-dibenzoyl-4-deoxystreptamine, 12.5 ml. of redistilled acetic acid (b.p. 118°), and 12.5 ml. of concentrated nitric acid was heated using a steam bath for 6 hr. Brown fumes appeared after heating for ca. 5 min. The solution was evaporated to dryness; 30 ml. of water was added to the residue and the mixture was extracted five times with a total of 100 ml. of chloroform. The chloroform extracts were evaporated to dryness; this gave 0.291 g. (0.00238 mole), 88%, of benzoic acid (infrared spectrum identical to that of an authentic sample).

The aqueous layer was evaporated to dryness; this gave 0.137 g. of a gummy solid (faint positive ninhydrin, negative Nessler test). A paper chromatogram run in EW showed only a faint, blue spot at R_F 0.69. Samples of aspartic acid and glycine run on the same chromatogram showed

a purple spot, R_F 0.25, and a red-purple spot, R_F 0.29 respectively.

2,4-Dibenzamido-3-hydroxyglutaraldehyde

The method of Carter, et al., (42) was used to prepare 2,4-dibenzamido-3-hydroxyglutaraldehyde. From 5.0 g. (0.013 mole) of N,N'-dibenzoylstreptamine was obtained 3.20 g., 70%, of white crystals, m.p. 132-133° [lit. (42) m.p. 130-131°]. Also obtained was 0.52 g., 12%, of white crystals, m.p. 164-166° [lit. (42) m.p. 164-165°], thought by the original workers to be another form of the compound.

2,4-Dibenzamido-3-hydroxyglutaric Acid

The procedure described by Carter, Loo, and Rothrock (42) was used to prepare 2,4-dibenzamido-3-hydroxyglutaric acid. From 4.69 g. (0.0132 mole) of 2,4-dibenzamido-3-hydroxyglutaraldehyde was obtained 162 g., 32%, of white crystals, m.p. 192-193° [lit. (42) m.p. 199-200°].

An attempt was made to prepare the acid by oxidation of the aldehyde with Tollen's reagent. Tollen's reagent was prepared by dissolving 0.30 g. (0.0018 mole) of silver nitrate (Baker 3426, used as received) in 5 ml. of water and adding ammonium hydroxide dropwise until all the silver oxide formed just dissolved.

The Tollen's reagent was added dropwise to a solution composed of 0.200 g. (0.000562 mole) of 2,4-dibenzamido-3-hydroxyglutaraldehyde and 300 ml. of absolute ethanol. The solution was left in darkness for 24 hr. Silver was present in the flask both as a black precipitate and as a mirror. The mixture was evaporated to dryness and 40 ml. of water was added to the residue. The mixture was filtered and the residue was washed with 40 ml. of absolute ethanol. The alcohol washings were evaporated; this

gave 0.157 g. of brown residue. The aqueous filtrate was acidified with dilute hydrochloric acid and the mixture was filtered. The filtrate was evaporated to dryness; this gave 0.237 g. of orange crystals and gum. The residue was treated with 15 ml. of 95% ethanol and the mixture was filtered. The residue was inorganic and weighed 0.040 g. The filtrate gave 25 mg. of white wax upon evaporation. The wax was water-soluble and gave a positive ninhydrin test. A blank Tollen's reaction carried out under identical conditions gave no silver mirror or precipitate.

2,4-Dibenzamido-3-hydroxyadipaldehyde

To a solution of 0.225 g. (0.000608 mole) of N,N'-dibenzoyl-4-deoxystreptamine and 200 ml. of water was added 0.130 g. (0.000608 mole) of sodium metaperiodate (Baker 3756, used as received). The solution was left at ca. 25° for 40 hr. and then lyophilized. The residue was triturated with ca. 125 ml. of acetone without heating. The filtrate was evaporated at ca. 25°; this gave 0.216 g., 96%, of white crystals, m.p. 142-144° [lit. (22) m.p. 148-149°].

Attempted Oxidation of 2,4-Dibenzamido-3-hydroxyadipaldehyde to 2,4-Dibenzamido-3-hydroxyadipic Acid

Bromine Water

To a suspension of 0.169 g. (0.000460 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde in 85 ml. of water was added ca. 0.3 ml. (0.0055 mole) of bromine (Matheson, Coleman and Bell BX965, used as received). The solution was left at room temperature for 80 hr., and a stream of air was then bubbled through the solution to remove the excess bromine. The colorless solution was heated to boiling and 1 g. of calcium carbonate

was added. The mixture was filtered and the filtrate was evaporated to ca. 1 ml. The addition of 10 ml. of absolute ethanol caused no precipitation. The solution was evaporated to dryness and 20 ml. of water was added to the residue. The solution was heated to boiling and 0.12 g. (0.00095 mole) of oxalic acid dihydrate was added. The mixture was filtered and the filtrate was evaporated to ca. 1 ml. A purple gum formed together with a tan solid. The solid was filtered. It weighed 0.034 g. and did not melt up to 300°. It was insoluble in sodium bicarbonate solution.

To a solution of 1 ml. (0.018 mole) of bromine and 250 ml. of water was added 0.500 g. (0.000136 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde. The suspension was stirred magnetically at room temperature for 90 hr., and the excess bromine was then removed by means of a stream of air. A 50 ml. aliquot of the solution was evaporated to dryness; this gave an orange liquid which overnight turned to a black tar. The remainder of the solution was treated with 3 g. of calcium carbonate, heated to boiling, and filtered. The solid material was completely inorganic. The filtrate was evaporated to ca. 3 ml.; this gave only 0.030 g. of a tan solid.

To a solution of 0.300 g. (0.000815 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde and 1 l. of water was added 3 g. (0.03 mole) of calcium carbonate and 0.6 ml. (0.01 mole) of bromine. The mixture was stirred mechanically at ca. 25° for 84 hr. with exclusion of light by means of aluminum foil. The excess bromine was then removed and the mixture was filtered; this gave 1.94 g. of calcium carbonate.

The filtrate was evaporated to ca. 5 ml. at a temperature not

exceeding 50°. When 50 ml. of absolute ethanol was added, a precipitate formed. The mixture was filtered; this gave 0.147 g. of tan solid. The solid was suspended in 40 ml. of boiling water and 0.215 g. (0.0017 mole) of oxalic acid dihydrate was added. The mixture was filtered; this gave 0.070 g. of white solid. To the filtrate was added two drops of 12N hydrochloric acid, and the solution was refrigerated overnight. Since no precipitate formed, the solution was concentrated to ca. 1 ml. by means of a stream of air. The mixture was filtered; this gave 0.012 g. of white solid, m.p., sublimed ca. 155°.

Catalytic Oxidation

A mixture of 0.105 g. (0.00125 mole) of sodium bicarbonate, 10 ml. of water, 10 ml. of acetone, and 1.0 g. of 5% platinum-on-carbon (Engelhard Industries, used as received) was equilibrated under 1 atm. of oxygen at ca. 25°. A solution of 0.200 g. (0.000545 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde and 7 ml. of acetone was added and the mixture was stirred for ca. 48 hr. There was no noticeable uptake of oxygen.

The mixture was filtered and the catalyst was washed with acetone and water. The combined filtrate and washings were evaporated to ca. 5 ml. at a temperature not exceeding 45°. About 5 mg. of black solid was removed by filtration. The filtrate was acidified to pH 1 with dilute hydrochloric acid. The solution was cooled in an ice-water bath; a black gum precipitated. The mixture was filtered; this gave a residue of ca. 5 mg. of black gum. The filtrate was neutralized by means of IR-45 ion-exchange resin (hydroxyl phase), and the solution was refrigerated. About 0.030 g. of white gum was removed by filtration. The filtrate was extracted with chloroform and the extract was evaporated; this gave ca.

0.015 g. of white gum. Infrared spectra in chloroform of the black solid, black gum, and last white gum all showed $\lambda_{max.}$ 5.60 and 6.00 μ, among others, but no peak between those wavelengths. The first white gum was insoluble in chloroform.

Sodium Hypoiodite

To a solution of 0.184 g. (0.000500 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde and 750 ml. of water was added a solution containing 0.515 g. (0.00203 mole) of iodine, 1.30 g. (0.0078 mole) of potassium iodide, and 40 ml. of water. To the dark solution that resulted was added 60 ml. of 0.1N sodium hydroxide over a 20 min. period. The yellow solution was stirred for ca. 10 min. and acidified to ca. pH 1 with 10% sulfuric acid. The solution was extracted with petroleum ether until no iodine remained in the aqueous layer, and the solution was then neutralized to ca. pH 7 with aqueous sodium bicarbonate. The solution was evaporated to dryness at a temperature not exceeding 45°. The white residue was leached with 75 ml. of hot absolute ethanol and the mixture was filtered. The insoluble material was completely inorganic. The filtrate was evaporated to dryness; this gave a yellow-white residue which was completely soluble in 2 ml. of water and gave only a very faint red color in the ninhydrin test. The solution was evaporated to dryness; the residue charred only very slghtly when subjected to an ignition test.

2,4-Dibenzamido-3-hydroxyadipaldehyde Tetraethyl Mercaptal

A suspension of 2.37 g. (0.00645 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde in ca. 1 kg. (16 mole) of ethanethiol (Eastman 958,

used as received) was saturated with hydrogen chloride gas; the solution was left at ca. 25° for 24 hr. The ethanethiol was removed by means of a stream of air and the gummy residue was dissolved in 25 ml. of chloroform. The solution was chromatographed on a 5.0 cm. diameter column packed with chloroform and 300 g. of acid-washed alumina (Merck 71695, used as received). Chloroform was used as the eluting agent.

The residue from fractions 1-5 (400 ml., 1.64 g. of yellow oil) was discarded. The residue from fractions 6-9 (200 ml., 0.47 g. of yellow gum) was saved for rechromatography. The residue from fractions 10-18 (1100 ml., 2.54 g. of white solid) was recrystallized from a mixture of 15 ml. of benzene and 15 ml. of petroleum ether. White crystals, m.p. 144-145° [lit. (22) m.p. 146-146.5°], that weighed 1.88 g. were obtained. The eluting agent was changed to 5% methanol-chloroform. The residue from fractions 19-23 (750 ml., 0.82 g. of white-brown solid) was recrystallized from a solution of 10 ml. of benzene and 10 ml. of petroleum ether. White crystals, m.p. 143-145°, that weighed 0.72 g. were obtained. The residue from fraction 24 (150 ml., 0.025 g. of white gum) was discarded. The total yield of material melting at ca. 145° was 2.60 g., 70%.

Attempted Preparation of 2,4-Dibenzamido-3-hydroxyhexane

Raney Nickel, Hydrogen

A mixture of 0.113 g. (0.000195 mole) of 2,4-dibenzamido-3-hydroxyadipaldehyde tetraethyl mercaptal, 15 ml. of 95% ethanol, and ca. 1.5 g. of Raney nickel No. 28 catalyst (Raney Catalyst Company, Chattanooga, Tenn., washed six times with water) was hydrogenated at ca. 25° for ca. 40 hr. under 1 atm. of hydrogen. The reaction yielded only starting material. Another attempt using 10 ml. of dioxane as solvent and a reaction

time of 20 hr. also yielded only starting material.

Raney Nickel, Boiling Ethanol

A mixture of 0.125 g. (0.000215 mole) of the tetraethyl mercaptal, 20 ml. of 70% ethanol, and ca. 5 ml. of moist Raney nickel was boiled under reflux for 2 hr. The reaction yielded only starting material.

Raney Nickel, 40 p.s.i. of Hydrogen

A mixture of 0.125 g. (0.000215 mole) of the tetraethyl mercaptal, 20 ml. of dioxane, and 10 ml. of Raney nickel was subjected to a pressure of 40 p.s.i. (gage pressure) of hydrogen for 24 hr. in a Parr Pressure Reaction Apparatus. The catalyst was removed by filtration and the filtrate was evaporated; this yielded a gummy residue. The residue was treated with 15 ml. of hot 95% ethanol and the mixture was filtered. The insoluble material consisted of 0.033 g. of white crystals that did not melt up to 330°. The filtrate was evaporated and yielded only starting material.

Raney Nickel, Boiling Ethanol, Mechanical Stirring

A mixture of 0.500 g. (0.000862 mole) of the tetraethyl mercaptal, 140 ml. of 70% ethanol, and ca. 25 ml. of Raney nickel was boiled under reflux for 48 hr. with mechanical stirring. The mixture was filtered while hot and the residue was washed with ca. 100 ml. of hot 90% ethanol. The combined filtrate and washings were evaporated to dryness. The residue was treated with 50 ml. of hot absolute ethanol and the mixture was filtered. The insoluble material consisted of white crystals that did not melt up to 330° and weighed 0.183 g. The filtrate, when evaporated, yielded 0.350 g. of starting material.

A small amount of the product that did not melt up to 330° was recrystallized from acetic acid. It then showed m.p. 252-254° (dec.).

Anal. $Ca(C_2H_3O_2)_2$ (158.2) Calcd.: C, 30.37; H, 3.82; residue, 34.45
Found : C, 29.29; H, 3.98; residue, 34.5

A black reaction using 50 ml. of Raney nickel, 280 ml. of 70% ethanol, and no tetraethyl mercaptal yielded 0.470 g. of white crystals which did not melt up to 330°.

An attempted reduction under identical conditions, except for a reaction time of seven days, gave only the starting material and the substance which does not melt up to 330°.

Hexaacetylstreptamine

To 1.23 g. (0.0045 mole) of streptamine sulfate was added 75 ml. of acetic anhydride (Matheson, Coleman and Bell P2788, redistilled, b.p. 139°) and 0.756 g. (0.00922 mole) of sodium acetate (Baker 3466, dried at 130° for 12 hr.). The mixture was boiled under reflux for 1 hr. and the acetic anhydride and acetic acid were removed by vacuum distillation. The residue was leached with 50 ml. of hot chloroform; the mixture was filtered and the filtrate was evaporated to dryness. White crystals that weighed 0.746 g., 35%, were obtained. The compound was recrystallized from a solution of 7 ml. of chloroform and 5 ml. of ether; this gave 0.620 g., 31%, of white crystals, m.p., partial melting at 250°, sublimed at 330-350° [lit. (11) m.p., partial melting at 250°; m.p. 342-345°, sealed capillary]. The infrared spectrum, determined as a potassium bromide pellet, is shown as Fig. 8. The n.m.r. spectrum of a 10% solution of the compound in deuterochloroform was determined.

For analysis a small amount of the compound was recrystallized from ethanol. The sample showed partial melting at 254-255°, but crystals quickly reformed and sublimed at 342-348°.

Anal. $C_6H_8N_2O_4(C_2H_3O)_6$ (430.4) Calcd.: C, 50.23; H, 6.06; N. 6.51

$C_6H_7N_2O_4(C_2H_3O)_7$ Calcd.: C, 50.84; H, 5.97; N, 5.93

Found : C, 51.34, 50.93; H, 6.26, 6.08; N, 5.99, 6.14

The residue from the leaching of the reaction mixture with hot chloroform was extracted with chloroform for eight days in a Sohxlet apparatus. The extracts yielded a total of 1.13 g., 59%, of white crystals, m.p., partial transition to longer needles at ca. 250°, needles sublimed at 330-345° [lit. (11) m.p., partial melting and transition to longer needles at 240-247°; m.p. 342-345° in a sealed capillary]. The infrared spectrum of the compound, determined as a potassium bromide pellet, is shown as Fig. 9.

For analysis a small amount of the compound was recrystallized from acetic acid. The melting point was unchanged.

Anal. $C_6H_8N_2O_4(C_2H_3O)_6$ (430.4) Calcd.: C, 50.23; H, 6.06; N, 6.51

Found : C, 49.92; H, 6.03; N, 6.56

The total yield of polyacetylstreptamine was 1.75 g., 94%.

N,N'-Diacetylstreptamine

The procedure described by Peck, Hoffhine, Peel, Graber, Holly, Mozingo, and Folkers (11) was used to prepare N,N'-diacetylstreptamine. From 0.475 g. (0.00110 mole) of hexaacetylstreptamine was obtained 0.230 g., 77%, of white crystals, m.p. 275-280° dec. [lit. (11) m.p. 283-284°].

Heptaacetylstreptamine, 0.184 g. (0.00043 mole), when treated similarly, yielded 0.075 g., 68%, of white crystals, m.p. 275-280° dec.

The infrared spectra of N,N'-diacetylstreptamine from both sources were identical and showed $\lambda_{max.}$ 2.80, 3.00, 5.85, 6.18 and 6.40 μ, among others.

Pentaacetyl-4-deoxystreptamine

A mixture of 2.00 g. (0.00541 mole) of N,N'-dibenzoyl-4-deoxystreptamine and 500 ml. of 6N hydrochloric acid was boiled under reflux for 24 hr. The solution was extracted five times with a total of 1 l. of chloroform. The chloroform extracts were combined and evaporated to dryness. The residue was recrystallized from 7 ml. of water; this gave 1.20 g. (0.00975 mole), 91%, of benzoic acid, m.p. 120-121° (sealed capillary).

The acidic solution was evaporated to dryness; the last traces of water were removed *in vacuo*. To the dry 4-deoxystreptamine dihydrochloride (theoretically 0.0054 mole) was added 100 ml. (1.06 mole) of redistilled acetic anhydride and 1.00 g. (0.012 mole) of dry sodium acetate. The mixture was boiled under reflux for 1 hr.; the acetic anhydride and acetic acid were then removed by vacuum distillation. The residue was dried *in vacuo* for 12 hr. and then leached with 100 ml. of hot chloroform. The mixture was filtered; this gave 0.73 g. of tan solid, which was discarded. When the filtrate was evaporated to dryness, 2.43 g. of white crystals was obtained. The compound was recrystallized from 50 ml. of absolute ethanol; this gave 0.713 g. of white crystals, m.p. 310-312°. A second crop weighed 0.115 g., m.p. 305-310° which brought the total yield to 0.828 g., 41%.

The infrared spectrum, determined as a potassium bromide pellet,

is shown as Fig. 10. For analysis a small amount of the compound was recrystallized twice from absolute ethanol. The sample melted at 319-320°.

Anal.	$C_{16}H_{24}N_2O_8$	Calcd.: C, 51.60; H, 6.50; N, 7.52
	(372.4)	Found : C, 51.39; H, 6.47; N, 7.48

N,N'-Diacetyl-4-deoxystreptamine

A solution of 0.712 g. (0.0019 mole) of pentaacetyl-4-deoxystreptamine and 180 ml. of methanol (distilled from magnesium turnings, b.p. 64°) was cooled to ca. 0° in an ice-water bath and then saturated with ammonia gas. The solution was left at ca. 25° for 3 hr. The solution was evaporated to dryness; the residue was triturated with 20 ml. of methanol and the mixture was filtered. The residue weighed 0.030 g., m.p. 297-298° dec. The filtrate was evaporated to one-half volume and refrigerated. White crystals, 0.085 g., were obtained when the mixture was filtered. A second crop weighed 0.035 g., m.p. 289-290° (dec.), bringing the total yield to 0.150 g., 32%. The infrared spectrum, determined as a potassium bromide pellet, is shown in Fig. 11.

For analysis a small amount of the compound was recrystallized from methanol. The sample melted at 291-292°.

Anal.	$C_{10}H_{18}N_2O_5$	Calcd.: C, 48.77; H, 7.36; N, 11.37
	(246.3)	Found : C, 48.50; H, 7.29; N, 11.15

Preparation of Cupra B*

The designation "Cupra B" has been given to a solution which contains 15.0 ± 0.1 g. (0.235 mole) of copper ion, 240 ± 5 g. (14.1 mole)

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* The author would like to thank John H. Dixon, Jr. for the preparation of this solution.

of ammonia, and 1 g. of glycerol per liter (43).

Cupra B was prepared by packing a 3 l. flask ca. three-fourths full of copper turnings (Will Corp., washed with dilute hydrochloric acid) and then covering the turnings with concentrated ammonium hydroxide containing 1 g. of glycerol per liter. A stream of air was passed first through a concentrated ammonium hydroxide solution and then through the reaction mixture. The concentration of copper ion in the solution was followed by titration of aliquots with 0.1M ethylenediaminetetraacetic acid (EDTA). After ca. 6 hr. the copper ion concentration was 17.0 g./l. and the solution was decanted from the copper turnings. Analysis showed 17.0 g./l. of copper and 140 g./l. of ammonia (49). Ammonia gas was bubbled into the solution until analysis showed 15.10 g./l. of copper and 242 g./l. of ammonia. The solution was refrigerated until used.

Optical Rotations of Methyl α-D-Mannopyranoside

The observed optical rotation at the sodium D line of a solution (c 0.57, water) of methyl α-D-mannopyranoside (Nutritional Biochemical Company, used as received) in a 10.0 cm. polarimeter tube was +0.43 ± 0.02° (corrected for air). Thus, the specific rotation, $[\alpha]_D^{29}$, was +74 ± 3° [lit. (50) $[\alpha]_D$ +78° (c 0.57, water)]. The observed optical rotation at 436 mμ of the same solution was +0.89 ± 0.04° corrected for air). Thus, the specific rotation, $[\alpha]_{436}^{29}$, was +153 ± 5° [lit. (50) $[\alpha]_{436}$ +147° (c 0.57, water)].

The observed optical rotation at 436 mμ of a solution (c 0.587, Cupra B) of methyl α-D-mannopyranoside in a 1.00 cm. quartz cell was +0.59 ± 0.07° (corrected for air and cell). The specific rotation,

$[\alpha]_{436}^{29}$, was +1005 ± 120° [lit. (50) $[\alpha]_{436}$ +1050 (c 0.55, Cupra B)]. The rotational shift* is thus (+857 ± 125°) 194.2/100 = +1665 + 243° [lit. (50) rotational shift +1752].

Optical Rotations of N,N'-Diacetyl-4-deoxystreptamine

The observed optical rotation at the sodium D line of a solution (c 0.971, water) of N,N'-diacetyl-4-deoxystreptamine in a 10.0 cm. polarimeter tube was +0.05 ± 0.02° (corrected for air). Thus the specific rotation, $[\alpha]_D^{29}$, was +5 ± 2°. The observed optical rotation at 436 mμ of the same solution was +0.05 ± 0.09° (corrected for air). Thus the specific rotation, $[\alpha]_{436}^{29}$, was +5 ± 9°.

The observed optical rotations at 436 mμ of a solution (c 0.880, Cupra B) of the compound in a 1.00 cm. quartz cell were -0.78 ± 0.08°, -0.82 ± 0.09°, and -0.88 ± 0.05° (independent measurements, corrected for air and cell). Thus the specific rotations, $[\alpha]_{436}^{29}$, -886 ± 90°, -932 ± 100°, and -1000 ± 55°, respectively.

The observed optical rotations at 436 mμ were determined for another solution (c 0.700, Cupra B) of the compound. The values found were -0.70 ± 0.15°, -0.65 ± 0.06°, and 0.75 ± 0.05° (corrected for air and cell). Thus the specific rotations, $[\alpha]_{436}^{29}$, were -1000 ± 214°, -928 ± 86°, and -1070 ± 70°, respectively.

The average value of the six independent measurements on the two Cupra B solutions was -970 ± 60°. Thus the rotational shift, calculated using this average value, was (-975 ± 69°) 246.3/100 = -2400 ± 170°.

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* $\Delta[M]_{\text{Cupra B}} = ([\alpha]_{436,\ \text{Cupra B}} - [\alpha]_{436,\ \text{water}})$ Mol.Wt./100

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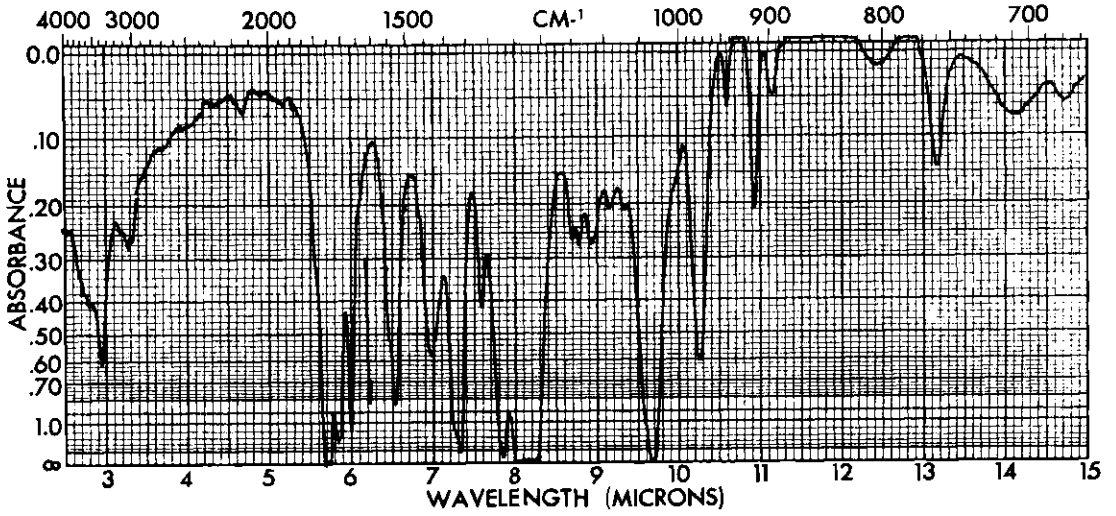

Fig. 8. The Infrared Spectrum of Heptaacetylstreptamine.

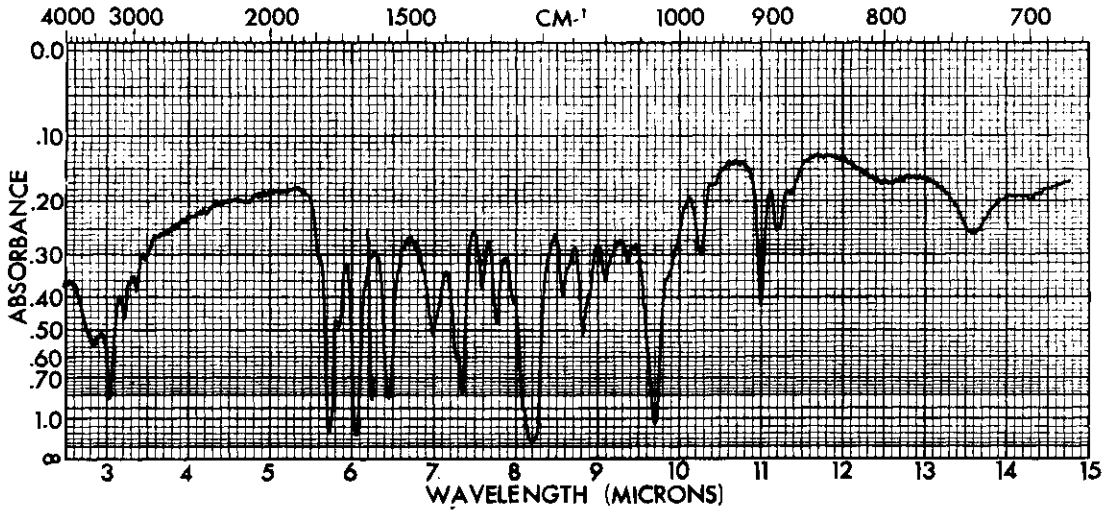

Fig. 9. The Infrared Spectrum of Hexaacetylstreptamine.

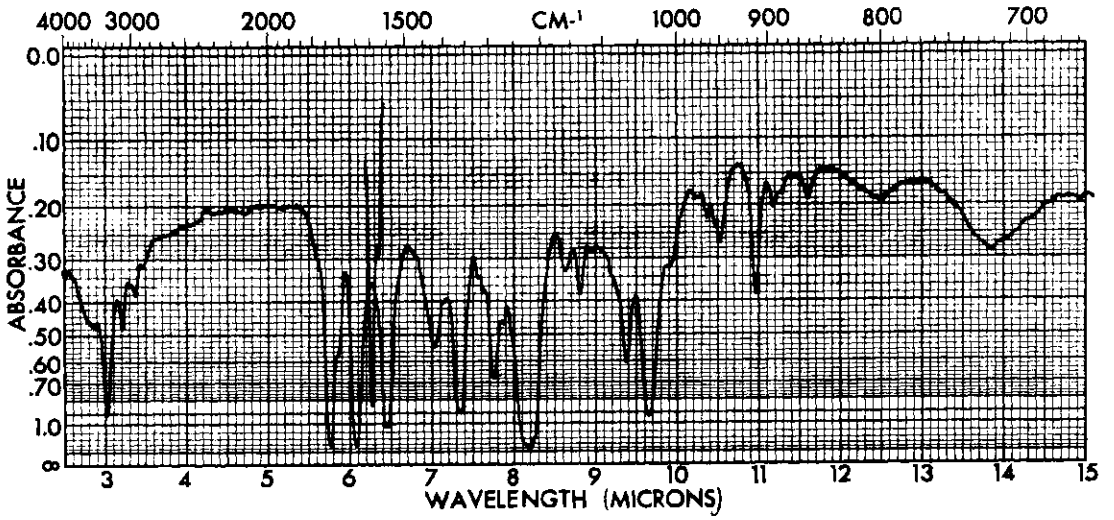

Fig. 10. The Infrared Spectrum of Pentaacetyl-4-deoxystreptamine.

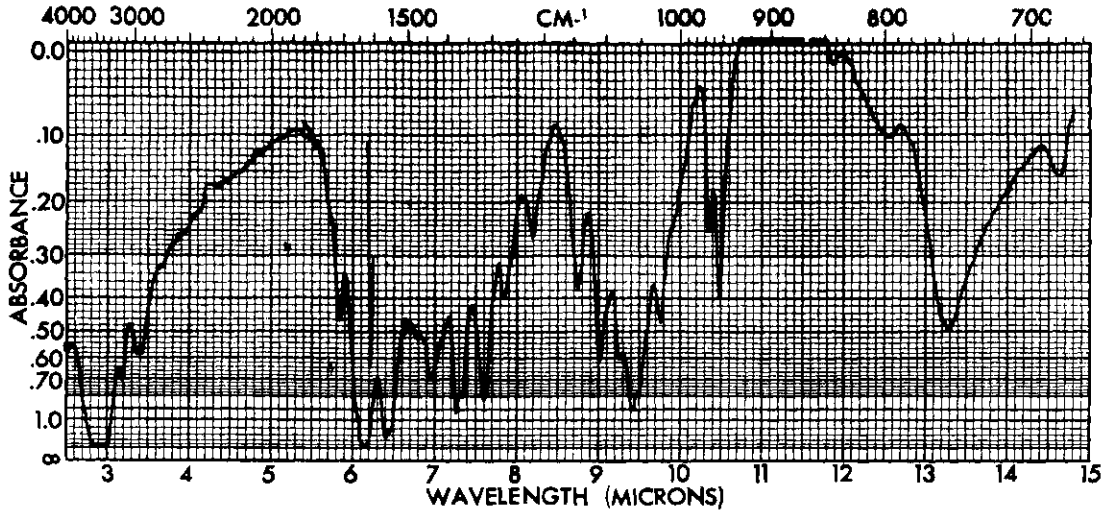

Fig. 11. The Infrared Spectrum of N,N'-Diacetyl-4-deoxystreptamine.

VITA

Aaron William Todd was born April 30, 1937, in Murfreesboro, Tennessee. He attended Crichlow Elementary School and Murfreesboro Central High School. He entered the Georgia Institute of Technology in September, 1955, and in June, 1959, received a Bachelor of Science degree, with honor, in chemistry. He began graduate study at the Georgia Institute of Technology in September, 1959, and held a National Science Foundation Cooperative Fellowship from that time until September, 1961. He was married on June 9, 1961, to Clara Patricia Wilson, and has two children, Roger Wilson and Christopher Lee.